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THE CARCINOGENIC HYDROCARBONS: CHEMICAL CONSTITUTION AND CARCINOGENIC ACTIVITY.

G. M. BADGER.*

From the Chemistry Department, University of Glasgow.

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IT is less than twenty years since it was first clearly demonstrated that certain pure polycyclic aromatic hydrocarbons have the property of inducing cancerous growths when applied, in solution, to the skin of animals. During the intervening years a very considerable number of synthetic cancer-producing substances has been prepared, and a very potent cancer-producing agent present in coal tar has been isolated in a pure state. For reviews see Cook, Haslewood, Hewett, Hieger, Kennaway, and Mayneord (1937); Cook and Kennaway (1938 and 1940); Fieser (1937 and 1938); Cook (1939 and 1943); Haddow (1947); Haddow and Kon (1947); Fieser, Fieser, Hershberg, Newman, Seligman and Shear (1937).

Apart from the polycyclic aromatic hydrocarbons, several other types of organic and inorganic compounds have the property of initiating cancers. The most important are: various azo compounds; certain amino stilbenes, and compounds related to 2-aminofluorene. Certain types of tumour have also been induced with oestrogenic hormones, with carbon tetrachloride, with ethyl carbamate ("urethane"), and by other substances. Hartwell (1941) has published a "Survey of compounds which have been tested for carcinogenic activity." This comprehensive work covers the literature through 1939, and includes data on 696 different chemical compounds, of which 169 were reported to be carcinogenic.

It is probably true to say that the first stage in the investigation of the carcinogenic substances is now over, and that future work must be directed towards the study of (*a*) the fate of such substances in the animal body, (*b*) the relationship, if any, between the known carcinogens and "spontaneous" cancer in humans, and (*c*) the mode of action of the carcinogens. In connection with the latter, the study of the relationship between chemical constitution and carcinogenic activity is of obvious importance. It was made clear from the early work that although relatively small changes in the structure of a carcinogen frequently converted it into an inactive derivative, major alterations could sometimes be carried out with but little change in activity. For this reason there was, at first, little or no attempt to produce an all-embracing theory. In recent years, however, there have been many attempts to find the relationship between chemical constitution and carcinogenic activity, and it appeared opportune to examine the available data on this subject. The present review is confined to the poly-

* I.C.I. Research Fellow.

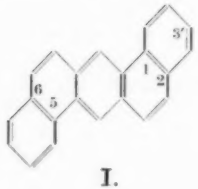
cyclic aromatic compounds, their heterocyclic analogues, and other closely related substances.

HISTORICAL.

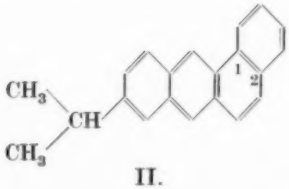
The first detailed investigations into the nature of the carcinogenic factor in coal tar were made by Bloch and Dreifuss (1921), who found that it is concentrated in the high boiling fractions, that it is free from nitrogen, arsenic and sulphur, and that it forms a stable picrate with picric acid. These results were extended by Kennaway (1924*a*, *b*, *c* ; 1925) and Kennaway and Sampson (1928), who prepared artificial tars both from complex organic materials, such as skin, hair, yeast and cholesterol, and from hydrocarbons, such as acetylene and isoprene. The latter tars were prepared by passing the hydrocarbon, with hydrogen, through a strongly heated tube. All this work indicated that the carcinogenic agent in tar is a complex aromatic hydrocarbon. Many of the known constituents of coal tar were accordingly tested by application of a solution of the pure hydrocarbon to the skin of mice ; but all the compounds tested were found to be inactive.

The first clue as to the nature of the carcinogenic factor was provided in 1927 by Mayneord, who observed that the same characteristic fluorescence bands were to be found in various carcinogenic tars. He found also that the complex mixture obtained by Schroeter by the action of aluminium chloride on tetrahydronaphthalene, which had no connection with any form of tar, and was carcinogenic (Kennaway, 1930), showed this same spectrum. Details of the fluorescence spectrum of several tar fractions and mineral oils, and of many pure compounds, were given by Hieger (1930). The fluorescence bands of the carcinogenic mixtures were found to be at 4000, 4180 and 4400A, and Hieger observed that pure 1:2-benzanthracene has a very similar spectrum, although the bands are shifted towards shorter wave lengths.

In 1929 Clar described the synthesis, by simple reactions, of a number of complex hydrocarbons related to 1:2-benzanthracene. These compounds were also prepared, in London, and submitted to biological test. Other benzanthracene derivatives were prepared by Cook in an attempt to reproduce exactly the fluorescence spectrum of the carcinogenic tars. As a result of this work 1:2:5:6-dibenzanthracene (I), 3′-methyl-1:2:5:6-dibenzanthracene and 6-*iso*propyl-1:2-benzanthracene (II) were found to be cancer-producing (Cook, Hieger, Kennaway and Mayneord, 1932 ; Clar, 1929 ; Cook, 1931, 1932*a*, 1932*b* ; Fieser and Dietz, 1929).



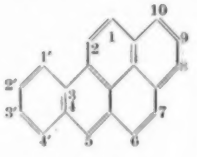
I.



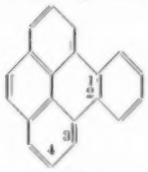
II.

Several other related compounds were also found to be cancer-producing, but none of these synthetic compounds was identical with the carcinogenic factor present in coal tar. All were found to give fluorescence bands more or less displaced from those observed with the carcinogenic tars. A strongly carcinogenic

crystalline fraction was separated from two tons of pitch by a lengthy series of purification processes, guided by a study of the fluorescence spectrum of each fraction (Hieger, 1937). From this fraction a compound was isolated (Cook, Hewett and Hieger, 1933), which was found to be identical with the new compound 3:4-benzpyrene (III) synthesized by Cook and Hewett. (In accordance with the " Patterson " system this compound was designated 1:2-benzpyrene in the original paper, but was later renamed 3:4-benzpyrene to conform to the older " Richter " system of numbering the pyrene molecule. Both systems of numbering pyrene are in common use at the present time. The Patterson system is used by both *Chemical Abstracts* and *British Abstracts*, but the older Richter system still appears to be the method of choice for most original journals.) The synthesis was achieved by condensing pyrene with succinic anhydride, reducing the



III.



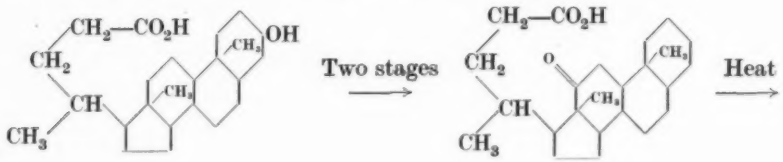
IV.

resulting keto acid to γ-1-pyrenylbutyric acid, cyclizing, reducing the resulting ketone, and finally, dehydrogenating. This method did not establish the structure rigidly, so the isomeric 1:2-benzyprene (designated 4:5-benzpyrene in the original paper) was also synthesized by the same series of reactions from *s*-hexahydropyrene. This latter synthesis can only lead to 1:2-benzpyrene, and as there are only two possible benzpyrenes, the structure of both products was confirmed. The identity of the synthetic 3:4-benzypyrene and the material isolated from coal tar was confirmed by mixed melting-point, by comparison of the fluorescence spectra, and it was also shown that both specimens were equally potent carcinogens.

The amount of 3:4-benzpyrene in coal tar has been variously estimated. According to Kruber (1940), 132 kg. of ordinary coal tar pitch contains 1 g. ; but Winterstein (1936) obtained 2·5 g. of almost pure 3:4-benzpyrene from 50 kg. of tar which contained 3 kg. of material boiling above 450°. According to Berenblum and Schoental (1943*a*), who used a spectrographic method of estimation, coal tar may contain as much as 1·5 per cent of 3:4-benzpyrene, and a simple method of extraction, by which Berenblum (1945*a*) isolated 75 mg. of almost pure material from 10 g. of crude coal tar distillate (b.p. 200–240°/0·1 mm.), has recently been devised.

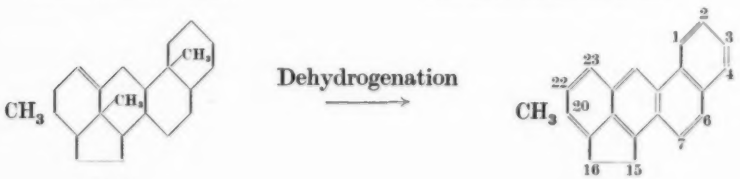
Recent work by Berenblum and Schoental (1947) has shown conclusively that coal tar contains carcinogens in addition to 3:4-benzpyrene. One of these is more potent to the rabbit's skin than to that of the mouse, and this evidently accounts for the fact that tumours in rabbits are more readily produced with coal tar than with 3:4-benzpyrene. These additional carcinogenic factors present in coal tar have not yet been isolated in a pure state, although there is little doubt that they also belong to the class of polycyclic aromatic hydrocarbons. In this connection it should be emphasized that the isolation of 3:4-benzpyrene was very greatly facilitated by the fact that this compound possesses a characteristic fluorescence which is many times more intense than that of the other compounds from which it has to be separated (Berenblum and Schoental, 1946*a*).

Following the discovery of the synthetic carcinogens, and of benzpyrene, attention was concentrated on the possibility that chemical carcinogens may play a part in the initiation of "spontaneous" human cancers, other than those known as "occupational" cancers. Kennaway and Cook (1932) suggested that carcinogenic polycyclic aromatic hydrocarbons may arise from certain sterols by some abnormal mechanism. Indeed, Cook (1933*b*) predicted that 20-methylcholanthrene (VIII), prepared by a series of reactions from deoxycholic acid (V) (Wieland and Dane, 1933; Cook and Haslewood, 1933, 1934, 1935) would prove to be carcinogenic before tests had been carried out. This prediction was substantiated, and methylcholanthrene was found to be a very potent cancer-producing substance (Barry, Cook, Haslewood, Hewett, Hieger and Kennaway, 1935). Methylcholanthrene has also been prepared from cholic acid, and from cholesterol. It was prepared synthetically by Fieser and Seligman (1935).



V.
(Deoxycholic acid.)

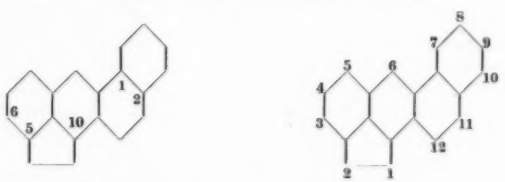
VI.
(12-Ketocholanic acid.)



VII.
(Dehydronorcholene.)

VIII.
(20-Methylcholanthrene.)

As it was first prepared from sterols, methylcholanthrene retains the numbering of the sterol ring system. It is clear, however, that it is most conveniently considered as a 1:2-benzanthracene derivative (A) substituted in positions 6, 5, and 10. In *Chemical Abstracts* methylcholanthrene is numbered as (B), and this method of numbering has been used in some original papers.



(A) (B)

In describing the biological results with methylcholanthrene, Barry *et al.* (1935) concluded: "Methylcholanthrene thus establishes a clear connecting

link between the carcinogenic hydrocarbons and the sterols and bile acids, and it is of great interest that the changes by which it is obtained from deoxycholic acid are all reactions of the type which are known to occur normally in the animal body, although there is no evidence that this particular sequence of changes involved in the formation of methylcholanthrene does actually occur in nature."

The possibility that chemical compounds of this type may play a part in the initiation of " spontaneous " human cancers remains a speculation, and a discussion of the evidence both for and against such an hypothesis is outside the scope of the present review. Carcinogenic factors, of unknown structure, do occur in human tissues, and this line of research is being actively pursued by several workers. For a review see Hieger (1947).

The discovery of methylcholanthrene further stimulated synthetic work, especially among di- and poly-substituted benzanthracenes. Other ring systems have also been investigated, and it is now clear that carcinogenic activity may be found in derivatives of many different ring systems, including many heterocyclic systems.

RELATIVE POTENCY OF CARCINOGENS.

Two techniques have been most extensively used for testing polycyclic aromatic hydrocarbons for carcinogenic activity. The first of these involves the application of the hydrocarbon, in 0·3 per cent solution in benzene, to the interscapular region of stock mice twice weekly (Cook, Hieger, Kennaway and Mayneord, 1932). Other solvents, including tetralin, xylene and acetone, have been used, and more dilute solutions are sometimes advantageous (Bachmann, Kennaway and Kennaway, 1938). Further, pure strain mice are sometimes preferred to stock mice. This method of test results, after a more or less prolonged latent period, in the appearance of papillomas and epitheliomas.

The second method, which gives rise to sarcomas, involves the injection of the carcinogen subcutaneously, or intraperitoneally. The crystalline material is sometimes injected in the form of a pellet of about 5 mg., but often, a solution of the hydrocarbon in lard, cholesterol, sesame oil, tricaprylin, or other such solvent, is injected. The quantity administered has varied from author to author ; and more than one injection has often been given, especially in the case of the less active compounds. Both stock mice and mice of pure strains have been used (Burrows, Hieger and Kennaway, 1932 ; Burrows, 1932 ; Shear, 1936*a*).

Both methods of administration sometimes result in the appearance of a number of tumours, e.g. of the liver, lungs, etc., not at the site of application. Such tumours are normally omitted from discussions of the relative potency of carcinogens of the polycyclic aromatic type—mainly because the data are insufficient for their correct appraisal (Badger, Cook, Hewett, Kennaway, Kennaway, Martin and Robinson, 1940). In the case of the carcinogenic azo compounds, and with certain other classes of compound not discussed in this review, tumours do not normally appear at the site of application, and the remote tumours are the only means of comparison of carcinogenic potency.

All carcinogens do not have the same potency. Tumours appear in treated animals (usually mice) only after a latent period, which varies from about a month or two for very active compounds, to nearly two years for the very feebly active compounds. Very active substances also induce tumours in a high percentage

of mice, while substances of slight activity produce tumours in only a few animals after a prolonged latent period.

Many attempts have been made to devise a system suitable for the accurate comparison of the potency of carcinogens. In many ways the best method is that due to Iball (1939), who introduced a *carcinogenic index*, defined by the relationship :

$$\text{Carcinogenic index} = \frac{\text{Percentage number of tumours}}{\text{Average latent period in days}}.$$

The percentage number of tumours was obtained from the number of animals bearing tumours, and the number of animals alive when the first tumour appeared. This method has the advantage that it eliminates animals which die too early to be affected by the compound under test. In this method of calculating the relative potency of carcinogens (and also in most other methods which have been suggested) papillomas are given the same weight as epitheliomas.

Fieser (1938) has also called attention to the importance of the latent period. He critically examined the data for the more important carcinogens, and attempted to compare the relative potencies by comparing average induction periods, weighted according to the number of tumours produced in any given experiment.

In a more recent study of the problem Berenblum (1945*b*) has suggested a system of *carcinogenic grades*, from XII to I, the grade XII being applied to compounds of very pronounced carcinogenic activity, and the grade I being applied to compounds with only trace activity. This system is also based on the latent period of carcinogenesis, and the carcinogenic grade is obtained from a diagram in which the grade (I to XII) is plotted against the latent period in weeks. The latter is on a logarithmic scale in order to eliminate the prominence which would otherwise be given to compounds of low potency.

Interesting discussions and information relative to the determination of carcinogenic potency are also to be found in papers by Shimkin (1940), Bryan and Shimkin (1940), and by Lea (1945). A comprehensive paper on the statistical treatment of measurements of the carcinogenic properties of tars and mineral oils has been published by Irwin and Goodman (1946).

None of these methods has been used extensively by other authors, and in only a very few papers has sufficient data been published for any of them to be used with reasonable accuracy. Iball's carcinogenic index has, however, been used in a few cases (Shimkin and Andervont, 1940 ; Badger, Elson, Haddow, Hewett and Robinson, 1942 ; Lacassagne, Buu-Hoï, Lecocq, and Rudali, 1946). Berenblum's carcinogenic grading is somewhat simpler, but in the opinion of the present author any attempt to compare the relative potency of different hydrocarbons by the use of twelve grades is apt to give a greater semblance of accuracy than is justified, especially when it is desired to compare the results from different laboratories, possibly using different animal strains and different techniques. All attempts to study the relationship between chemical constitution and biological activity must be limited by the inaccuracies inherent in all biological assays, and specific reference to the extreme difficulties in testing carcinogenic compounds has been made by Hartwell (1941, p. 9).

For these reasons the grading of potency in the present paper has been made

as simple as possible. The potency of the compounds to both epithelial cells and to cells of connective tissue have both been classified as follows :

++++	signifies	very marked carcinogenic activity.
+++	,,	marked carcinogenic activity.
++	,,	moderate carcinogenic activity.
+	,,	slight carcinogenic activity.
0	,,	inactive.

The working of the system in practice may be observed from Table I, in which the carcinogenic index of Iball, the carcinogenic grade of Berenblum, and the system adopted in the present paper are compared for 10 well-known compounds which have been tested by application to the skin of mice.

TABLE I.—*Relative Potency of Carcinogens to the Skin of Mice ; Comparison of Different Systems of Grading.*

Compound.	Per cent tumours.	Average latent period (days).	Iball's index.	Berenblum's grade.	This paper.
9:10-Dimethyl-1:2-benz-anthracene	65	43	151	X	++++
20-Methylcholanthrene	88·5	109	80	VIII	++++
3:4-Benzpyrene	89·5	119	75	VIII	++++
2-Methyl-3:4-benz-phenanthrene	75	155	48	VII	+++
10-Methyl-1:2-benz-anthracene	66·5	147	45	VI ?	+++
5-Methyl-1:2-benz-anthracene	87·5	317	28	V	++
1:2:5:6-Dibenzanthracene	63	239	26	VI	++
3:4-Benzphenanthrene	67	387	17	V	+
1:2:5:6-Dibenzacridine	24	350	7	4	+
3:4:5:6-Dibenzacridine	39·3	357	11	IV	+

This method of grading which is adopted has the very great advantage of simplicity. Even so, it is difficult, in some cases, to be certain of the most accurate grading, as the published data are often so meagre. Furthermore, in view of the difficulties of biological assay, the probable or expected error in grading is at least one + symbol.

THE STRUCTURE OF THE CARCINOGENIC COMPOUNDS.

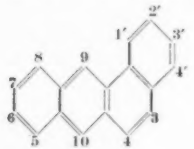
(a) *Polycyclic aromatic hydrocarbons, and the effect of methyl groups.*

As has already been pointed out, the resemblance between the fluorescence spectra of the carcinogenic tars and that given by pure 1:2-benzanthracene (IX) provided the first clue as to the nature of the active principle. It also provided the starting-point for the synthetic approach, which led to the testing of 1:2:5:6-dibenzanthracene, to the synthesis and testing of 6-*iso*propyl-1:2-benzanthracene, and of other compounds. Since then many of the homologues of the tricyclic, the tetracyclic, the pentacyclic and a few hexacyclic aromatic hydrocarbons have

been tested, and examples of each class of compound have been shown to possess cancer-producing activity. In this and in the subsequent sections references to tests for carcinogenic activity are to be found in the tables. Only in the cases of compounds not listed in tables have references to the literature been appended in the text.

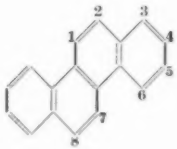
Anthracene, phenanthrene and a few simple derivatives were tested in the very early experiments (Hartwell, 1941) and found to be inactive. It is only recently that two simple derivatives have been shown to possess slight but definite carcinogenic activity to the skin of mice. These active homologues are 1:2:3:4-tetramethylphenanthrene, and 9:10-dimethylanthracene, and they are the simplest examples of carcinogen yet found among the polycyclic aromatic hydrocarbons (Badger, Cook, Hewett, Kennaway, Kennaway and Martin, 1942; Kennaway, Kennaway and Warren, 1942).

There are six possible tetracyclic aromatic hydrocarbons composed entirely of aromatic rings, and each has been investigated. Only 3:4-benzphenanthrene (X) has been shown to possess significant, if slight, activity. Tumours have occassionally been attributed to chrysene (XI), but these are probably due to impurities almost invariably associated with this hydrocarbon. 1:2-Benzanthracene (IX) has also given one or two tumours in very extensive tests, but it is doubtful if these are significant. Triphenylene (XII), pyrene (XIII) and naphthacene (XIV) appear to be inactive.



IX.
1:2-Benzanthracene.

X.
3:4-Benzphenanthrene.



XI.
Chrysene.

XII.
Triphenylene.

XIII.
Pyrene.

XIV.
Naphthacene.

All the twelve possible monomethyl derivatives of 1:2-benzanthracene have been prepared and tested, many of them both by application to the skin and by subcutaneous injection. There is no doubt that the introduction of a methyl group into one of the " favourable " positions has a very pronounced effect, and may give rise to a very potent cancer-producing substance. The most active derivatives are those in which a methyl group has been introduced into positions 10-, 9-, or 5-. The introduction of a methyl group into positions 6-, 7-, 8-, 3- or 4- serves to produce a moderately to very slightly active substance, but methyl groups in the angular ring, that is in positions 1'-, 2'-, 3'- or 4'-, do not seem to

have this effect. 4′-Methyl-1 : 2-benzanthracene may have trace activity, but 1′-methyl-, 2′-methyl-, and 3′-methyl-1:2-benzanthracenes appear to be entirely inactive (Table II).

The effect of additional methyl groups is, in general, to give rise to more potent compounds. As a general rule, dimethylbenzanthracenes are more potent than either of the monomethyl derivatives to which they are related. 9:10-Dimethyl-1:2-benzanthracene is especially active to the skin of mice, and produces many tumours within a remarkably short latent period. 5:9-Dimethyl- and 5:10-dimethyl-1:2-benzanthracenes are also remarkably active cancer-producing substances, and 5:6-dimethyl-1:2-benzanthracene is also more active than either of the monomethyl derivatives to which it is related. On the other hand, there appear to be some exceptions to this generalization. 5:8-Dimethyl- and 3:9-dimethyl-1:2-benzanthracenes appear to be inactive, at least when administered by injection. Furthermore, it is also of some interest that all the dimethylbenzanthracenes having a methyl group in the angular ring are entirely inactive.

A few polysubstituted benzanthracenes have also been examined. 5:9:10-Trimethyl- and 6:9:10-trimethyl-1:2-benzanthracenes are extremely active carcinogens, as might be expected. On the other hand, 5:6:9:10-tetramethyl-1:2-benzanthracene appears to be somewhat less active than either of the trimethyl, *meso*-substituted benzanthracenes to which it is related. It is possible that there is an optimum number of methyl groups which can be introduced and which lead to an increase in potency. This possibility, though widely accepted, has not been extensively investigated.

The cholanthrenes, and related compounds, are most conveniently considered as substituted benzanthracenes, although the sterol numbering often used tends to confuse the relationship. Cholanthrene, 20-methylcholanthrene, 22-methyl- and 23-methylcholanthrene have been prepared and tested. They may be considered as benzanthracenes substituted in positions 10-, 5- and one other position. All were found to be very active, but there is little doubt that 20-methylcholanthrene is the most active, then cholanthrene, then 22-methylcholanthrene, and then least active, 23-methylcholanthrene. Law and Lewisohn (1941) have also examined these compounds by subcutaneous injection in " strain C " mice, and observed that 20-methylcholanthrene and 22-methylcholanthrene are significantly more potent in inducing lung tumours than the parent hydrocarbon, cholanthrene, and that 23-methylcholanthrene is significantly less potent than cholanthrene.

In view of the special interest of methylcholanthrene many related compounds were tested. One line of approach was to vary the positions of attachment of the dimethylene bridge. Acenaphthanthracene (4′:3-ace-1:2-benzanthracene) is only slightly active, 8:9-ace-1:2-benzanthracene is moderately active, 4:10-ace-1:2-benzanthracene is a potent carcinogen, but cholanthrene (5:10-ace-1:2-benzanthracene) is the most potent. These results again indicate the pre-eminence of positions 5- and 10- for the development of carcinogenic activity. It is surprising that 7-methyl-8:9-ace-1:2-benzanthracene is inactive.

It was at first thought that the dimethylene bridge might have some special significance for the development of carcinogenic activity, but this has since been disproved. 5:10-Dimethyl-1:2-benzanthracene, and cholanthrene are approximately equally potent; similarly, 4:10-ace-1:2-benzanthracene and 4:10-dimethyl-1:2-benzanthracene are moderately, and about equally, potent. The dimethylene

bridge present in these compounds therefore appears to be approximately equivalent to two methyl groups in the same positions.

There are six possible monomethyl derivatives of 3:4-benzphenanthrene, and five of these have been prepared and tested. 5-Methyl-3:4-benzphenanthrene has only recently been prepared (Newman and Wheatley, 1948). It is a remarkable feature of the benzphenanthrenes, which has not yet been explained, that these compounds are all very much less effective in producing cancer when administered by injection than when applied to the skin. 3:4-Benzphenanthrene (X) itself is slightly active to the skin of mice, but has proved ineffective when administered subcutaneously. 2-Methyl-3:4-benzphenanthrene is a potent carcinogen when applied to the skin, but is only very slightly active when administered by injection. 1-Methyl-3:4-benzphenanthrene is moderately active to the skin, and seems to be inactive by injection. The remaining monomethyl derivatives are only slightly active. Only a few dimethyl derivatives have been tested. These include 6:7-dimethyl-, and 2:9-dimethyl-3:4-benzphenanthrene, which have been shown to be inactive by injection ; but since these compounds have not been tested on the skin no inferences can be drawn.

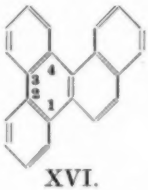
All the monomethyl chrysenes have been prepared (Bachmann and Edgerton, 1940), but all do not appear to have been tested. Work in this field is sometimes difficult to follow, as two systems of numbering the chrysene molecule are about equally used (Table IV). In America the Patterson system is used, but in Europe the Richter system is still commonly used, and is used in this report. The Patterson numbers are given in parenthesis. 1-Methylchrysene (5-) is a potent carcinogen, and 2-methyl-(6-), and 6-methylchrysene (4-) are slightly active. These results apply only to tests by subcutaneous injection, and since there is some evidence that the alkylchrysenes, like the alkylbenzphenanthrenes, are more potent when applied to the skin, the relative carcinogenic activities of these derivatives should be accepted with caution. 1:2-Dimethylchrysene (5:6-), for example, is a moderately active carcinogen to the skin of mice, but only very feebly active when administered by injection. 6:7-Dimethylchrysene (4:5-) and 6:7-methylenechrysene (4:5-) are feebly active by injection, but have not been tested by application to the skin.

Relatively little work has been done on the homologues of triphenylene, naphthacene, and pyrene. Triphenylene itself is not carcinogenic, and the 1-methyl- and 1:4-dimethyl-derivatives also appear to be inactive, at least when administered by injection (Shear and Leiter, 1941). Naphthacene derivatives are very readily photo-oxidized, and are not therefore very suitable for test (23rd Annual Report, *B.E.C.C.*, 1946, p. 109). 2-*iso*Propylnaphthacene has been tested for comparison with its cancer-producing isomer, 6-*iso*propyl-1:2-benzanthracene : it did not induce tumours (Barry *et al.*, 1935). Pyrene and 4-methylpyrene are also inactive (Barry *et al.*, 1935 ; Badger *et al.*, 1940).

There are fifteen possible pentacyclic aromatic hydrocarbons composed entirely of benzene rings, and all have been tested for cancer-producing activity (Barry *et al.*, 1935). 1:2:5:6-Dibenzanthracene (I) was the first pure synthetic substance shown to be carcinogenic ; 1:2:7:8-dibenzanthracene has given a few tumours in very extensive tests, and is only very feebly active ; 1:2:3:4-dibenzanthracene seems to be inactive. 3:4-Benzpyrene (III) is a very potent carcinogen, but 1:2-benzpyrene (IV) is inactive. 1:2:5:6-Dibenzphenanthrene (XV) and 1:2:3:4-dibenzphenanthrene (XVI) are moderately active, and the remaining

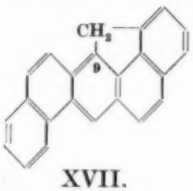
pentacyclic hydrocarbons are inactive. It is interesting that of the fifteen possible pentacyclic hydrocarbons, thirteen may be considered as derived from phenanthrene, and only five of these have given tumours.

XV.

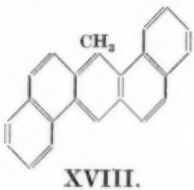


XVI.

A few homologues of 1:2:5:6-dibenzanthracene have been examined with interesting results. 2′-Methyl-, 3′-methyl-, and 4-methyl-1:2:5:6-dibenzanthracenes are all weakly to moderately active (Cook, 1932*b*; Barry *et al.*, 1935). 1′:9-Methylene-1:2:5:6-dibenzanthracene (XVII) is moderately active (Shear, 1936*a*); but 9-methyl-1:2:5:6-dibenzanthracene (XVIII) is an extremely potent carcinogen (Shear, Leiter and Perrault, 1940). It is therefore surprising that 9:10-dimethyl-1:2:5:6-dibenzanthracene is only very feebly active, having pro-



XVII.



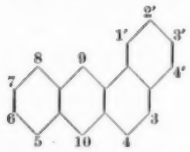
XVIII.

duced only one papilloma. This result, which appears to run contrary to the 1:2-benzanthracene series, was observed in the early work (Cook, 1932*b*), and materially delayed the recognition of the importance of the *meso* substitution in the related series. It is also noteworthy that although 9:10-dimethyl-1:2:3 4-dibenzanthracene is inactive, as might be expected, 9:10-dimethyl-1:2:7:8-dibenzanthracene is a potent cancer-producing substance (23rd Annual Report, *B.E.C.C.*, 1946, p. 107). These observations have been offered in support of the hypothesis linking carcinogenic activity with an activated phenanthrene-type bond (Haddow, 1947).

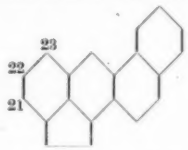
The effect of methyl groups on the activity of 3:4-benzpyrene is also of considerable interest as, here again, the effect seems to run counter to that which might be expected from a consideration of the methylbenzanthracenes. 2′-Methyl- and 3′-methyl-3:4-benzpyrenes appear to be inactive, at least when administered by injection, although these positions of substitution correspond to the 7- and 6- positions in benzanthracene. Furthermore, 4′-methyl- and 6-methyl-3:4-benzpyrene are considerably less active than the parent hydrocarbon (III). Again, 9-methyl-3:4-benzpyrene and 5-methyl-3:4-benzpyrene appear to have the same activity as the parent hydrocarbon. The 9- position corresponds to the 3′- position in benzanthracene, and 3′-methyl-1:2-benzanthracene is inactive.

Only a few examples of hexacyclic aromatic hydrocarbons have been examined, Both 1:2:3:4-dibenzpyrene and 3:4:8:9-dibenzpyrene (XIX and XX) are active,

TABLE II.—*Methylbenzanthracenes, Acebenzanthracenes and Cholanthrenes.*



Benzanthracene numbering.*



Cholanthrene numbering.†

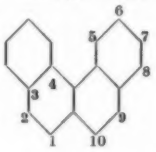
Compound.	Carcinogenic activity.			
	Skin.	References.	Subcutaneous tissue.	References.
1:2-Benzanthracene	0	a	0	e
1′-Methyl-	0	b	0	b, c, e
2′-Methyl-	0	a	..	..
3′-Methyl-	0	a	..	..
4′-Methyl-	0	b	..	..
3-Methyl-	+	a, b	++	b
4-Methyl-	+	a	++	e
5-Methyl-	++	a	++	d
6-Methyl-	+	a	..	..
7-Methyl-	+	a, b	+	b, d
8-Methyl-	+	b	0	e
9-Methyl-	++	b	+++	d
10-Methyl-	+++	b	++++	d
1′:10-Dimethyl-	..	..	0	f
2′:6-Dimethyl-	0	a, g	0	a, g
2′:7-Dimethyl-	0	a	..	..
3′:6-Dimethyl-	0	a	..	..
3′:7-Dimethyl-	0	a	..	..
3:9-Dimethyl-	..	..	0	e
4:9-Dimethyl-	..	..	+++	e
4:10-Dimethyl-	..	..	++	f
5:6-Dimethyl-	+++	a	..	..
5:8-Dimethyl-	..	..	0	h
5:9-Dimethyl-	..	..	++++	d
5:10-Dimethyl-	..	..	++++	d
6:7-Dimethyl-	+	a	..	..
8:10-Dimethyl-	..	..	+++	h
9:10-Dimethyl-	++++	b	+++	b, d, f, h
4′:3-Ace-	+	b	++	b
4:10-Ace-	..	..	+++	d, h
5:10-Ace-(Cholanthrene)	++++	j	++++	k, l
8:9-Ace-	..	..	++	k
5:9:10-Trimethyl-	++++	b, m	+++	b
6:9:10-Trimethyl-	++++	b, g	++	b
20-Methylcholanthrene	++++	j	++++	l
22-Methylcholanthrene	..	..	++++	l
23-Methylcholanthrene	..	..	++++	l
7-Methyl-8:9-ace-	..	..	0	k
5:6:9:10-Tetramethyl-	+++	b	+	b

and so also is 7-methyl-1:2:3:4-dibenzpyrene. All the other hexacyclic aromatic hydrocarbons which have been tested have failed to give rise to tumours (Kleinenberg, 1938, 1939, 1940; Bachmann, Cook, Dansi, de Worms, Haslewood, Hewett and Robinson, 1937).

XIX.

XX.

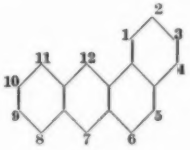
TABLE III.—*Methylbenzphenanthrenes.*



Compound.	Carcinogenic activity.			
	Skin.	References.	Subcutaneous tissue.	References.
3:4-Benzphenanthrene . .	+	a, c	0	c, d
1-Methyl-	++	c	0	c
2-Methyl-	+++	b, c	+	c, d
6-Methyl-	+	c	+	c
7-Methyl-	+	c	0	c
8-Methyl-	+	c	0	c
2:9-Dimethyl-	..	..	0	d
6:7-Dimethyl-	..	..	0	e

a, Barry, Cook, Haslewood, Hewett, Hieger and Kennaway (1935); b, Bachmann, Cook, Dansi, de Worms, Haslewood, Hewett and Robinson (1937); c, Badger, Cook, Hewett, Kennaway, Kennaway, Martin and Robinson (1940); d, Shear and Leiter (1941); e, Shear (1938).

* This method of numbering has been used most extensively in both the chemical and boilogical literature. It is not, however, the system which is recommended by Patterson. The Patterson numbering (A) is used in *Chemical Abstracts*, and has also been used recently in the *Journal* of the American Chemical Society.

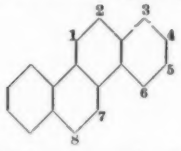


(A).

† This is the "sterol" numbering for cholanthrene. For the other systems see footnote to formula VIII.

References:—a, Barry, Cook, Haslewood, Hewett, Hieger and Kennaway (1935); b, Badger, Cook, Hewett, Kennaway, Kennaway, Martin and Robinson (1940); c, Shear (1939); d, Shear (1938); e, Shear and Leiter (1941); f, Shear, Leiter and Perrault (1940); g, Badger, Cook, Hewett, Kennaway, Kennaway and Martin (1942); h, Dunlap and Warren (1946); i, Shear and Perrault (1939); j, Bachmann, Cook, Dansi, de Worms, Haslewood, Hewett and Robinson (1937); k, Shear (1936*b*); l, Bradbury, Bachmann and Lewisohn (1941); m, Hartwell and Stewart (1942).

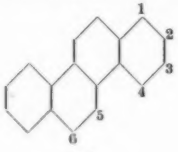
TABLE IV.—*Methylchrysenes.*



"Richter" numbering.*

Compound.	Carcinogenic activity. Skin.	References.	Subcutaneous tissue.	References.
Chrysene	0	a	0	c
1-Methyl-	..	..	+++	b
2-Methyl-	..	..	+	c
6-Methyl-	..	..	+	b
4:5-Dimethyl-	..	..	0	d
6:7-Dimethyl-	..	..	+	b
1:2-Dimethyl-	++	e	+	b, e

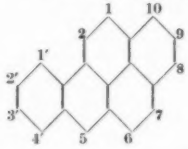
* This system of numbering is used in Europe, but the Patterson system (A) is used in America.



(A)

a, Bachmann, Cook, Dansi, de Worms, Haslewood, Hewett and Robinson (1937); b, Dunlap and Warren (1943); c, Shear and Leiter (1941); d, Shear (1938); e, Badger, Cook, Hewett, Kennaway, Kennaway, Martin and Robinson (1940).

TABLE V.—*Methylbenzpyrenes.*



Compound.	Carcinogenic activity. Skin.	References.	Subcutaneous tissue.	References.
3:4-Benzpyrene	++++	a, g	++++	a, h
2'-Methyl-	..	..	0	c
3'-Methyl-	0	b, f, i	0	c
4'-Methyl-	..	..	++	d
5-Methyl-	..	..	++++	e
6-Methyl-	..	..	++	c
9-Methyl-	..	..	++++	c

a, Fieser (1938); b, Schürch and Winterstein (1935); c, Fieser and Heymann (1941); d, Shear and Perrault (1939); e, Shear, Leiter and Perrault (1940); f, Winterstein, Vetter and Schön (1935); g, Barry, Cook, Haslewood, Hewett, Hieger and Kennaway (1935); h, Shear (1936*a*); i, Fieser and Hershberg (1938*b*).

(b) *Alkyl derivatives in homologous series.*

There appears to be little doubt that the substitution of one or more methyl groups into a " favourable " position of certain polycyclic aromatic hydrocarbons can transform a non-carcinogenic hydrocarbon into a carcinogenic derivative, or can materially increase the activity of a carcinogen. It was therefore of interest to study the effect of the higher alkyl groups.

The 5-*n*-alkyl-1:2-benzanthracenes have been extensively investigated, for all the members of the series as far as the *n*-heptyl derivative have been examined, most of them by both injection, and painting on the skin. When tested by the latter method all the compounds are active, but there is a progressive diminution in potency as the number of carbon atoms in the alkyl chain increases, 5-methyl-1:2-benzanthracene being moderately active, but the 5-*n*-heptyl derivative being only very slightly so. A plot of Iball's carcinogenic index for each compound against the number of carbon atoms in the alkyl chain has been published by Badger, Elson, Haddow, Hewett and Robinson (1942). When tested by subcutaneous injection, however, the higher members of the series (butyl, amyl, hexyl, and heptyl) failed to give tumours. 5-Methyl-1:2-benzanthracene is moderately active by this method, but comparable experiments with the ethyl and *n*-propyl derivatives do not appear to have been undertaken. 5-*iso*Propyl-1:2-benzanthracene was found to be moderately active to the skin, and slightly active by injection.

Of the 10-*n*-alkyl-1:2-benzanthracenes the methyl derivative is extremely potent, both by application to the skin and by injection. The ethyl homologue is rather less active when tested by injection, and the higher homologues have failed to give tumours when administered by this method. As these compounds have not been tested by application to the skin, it is difficult to decide whether activity is more easily lost in the 10- substituted series or in the 5- substituted compounds. It is of some interest, however, that 10-*iso*propyl-1:2-benzanthracene has been tested by both methods and appears to be completely inactive, while the 5-*iso*propyl- derivative, which has also been tested by both methods, is moderately active.

Little work has been carried out on the synthesis and testing of other homologous series of alkylbenzanthracenes. An apparent exception, however, is 6-*iso*propyl-1 : 2-benzanthracene, which appears to be more active than the methyl derivative ; but this comparison has been made only on the skin of mice, and not by injection. 3-*iso*Propyl- and 7-*iso*propyl-1:2-benzanthracenes have also been tested on the skin and found to be inactive ; the corresponding methyl derivatives are very slightly active. 20-Methylcholanthrene is rather more potent than the parent hydrocarbon, cholanthrene ; the higher members of this series, namely, the ethyl-, *iso*propyl-, and *tert.*-butylcholanthrenes are progressively less potent when tested by injection.

A short series of 2-alkyl-3:4-benzphenanthrenes has also been examined. In skin-painting experiments the 2-methyl- derivative proved very potent, and the 2-ethyl- and 2-*iso*propyl- derivatives were found to be less active. The benzphenanthrenes always show greater activity when tested on the skin than when given by injection, and the present series is no exception ; 2-methyl-3:4-benzphenanthrene was found to be only feebly active by this latter method, and the 2-*iso*propyl- derivative failed to give any tumours.

TABLE VI.—*Alkyl Derivatives in Homologous Series.*

Compound.	Carcinogenic activity. Skin.	References.	Subcutaneous tissue.	References.
5-Methyl-1:2-benzanthracene	++	a	++	e
5-Ethyl-	++	b	..	..
5-*n*-Propyl-	++	b	..	..
5-*iso*Propyl-	++	c	+	c
5-*n*-Butyl-	++	c	0	c
5-*n*-Amyl-	++	c	0	c
5-*n*-Hexyl-	+	c	0	d
5-*n*-Heptyl-	+	d	0	c
10-Methyl-1:2-benzanthracene	+++	c, f	++++	f
10-Ethyl-	..	..	+++	f
10-*n*-Propyl-	..	..	0	f
10-*iso*Propyl-	0	a	0	f
10-*n*-Butyl-	..	..	0	f
10-*n*-Amyl-	..	..	0	f
20-Methylcholanthrene	++++	b	++++	e
20-Ethylcholanthrene	..	..	+++	e, g
20-*iso*Propylcholanthrene	..	..	++	g
20-*t*-Butylcholanthrene	..	..	+	g
2-Methyl-3:4-benzphenanthrene	+++	c	+	c
2-Ethyl-	++	c, d	..	..
2-*iso*Propyl-	++	c	0	c
2-*n*-Propyl-	++	d	0	d

a, Barry, Cook, Haslewood, Hewett, Hieger and Kennaway (1935); b, Bachmann, Cook, Dansi, de Worms, Haslewood, Hewett and Robinson (1937); c, Badger, Cook, Hewett, Kennaway, Kennaway, Martin and Robinson (1940); d, Badger, Cook, Hewett, Kennaway, Kennaway and Martin (1942); e, Shear (1938); f, Shear, Leiter and Perrault (1940); g, Shear, Leiter and Perrault (1941).

(c) *The effect of other substituents.*

Although most of the early work was concentrated on an examination of hydrocarbons, a few derivatives carrying functional substituents were examined. 9-Amino-1:2:5:6-dibenzanthracene, and 9-methoxy-1:2:5:6-dibenzanthracene, for example, were found to be moderately active. On the other hand, the introduction of other substituents at the same position gave rise to inactive derivatives; these included the 9-nitro-, 9-hydroxy-, 9-acetoxy-, and 9-*n*-butyrylamino- substituted 1:2:5:6-dibenzanthracenes (Barry *et al.*, 1935). As has been pointed out above, 9-methyl-1:2:5:6-dibenzanthracene is a potent carcinogen, more active than the parent hydrocarbon (Shear, Leiter and Perrault, 1940).

Several substituted methylcholanthrenes have been tested (Shear, Leiter and Perrault, 1941). These include the 2-hydroxy-, 2-methoxy-, 3-hydroxy-, and 3-methoxy- derivatives, all of which are inactive. The effect of substitution on the dimethylene bridge is not so striking, however, for 15-hydroxy-20-methyl-

cholanthrene is still moderately active, and the corresponding keto derivative is slightly active. 6-Chloro- and 6-cyano-20-methylcholanthrenes are inactive.

In the above cases the effect of the substituent (other than methyl) has been to reduce the potency of the original hydrocarbon, and in some cases to abolish the activity altogether. Several functional derivatives of 9:10-dimethyl-1:2-benzanthracene have been examined, however, and in these cases the activity of the parent hydrocarbon seems to be retained. 5-Bromo- and 5-cyano-9:10-dimethyl-1:2-benzanthracenes have about the same activity as the parent dimethylbenzanthracene (Dunlap and Warren, 1946).

Substitution in the methyl groups of this hydrocarbon does reduce the potency. 9-Methyl-10-ethoxymethyl-, 9-methyl-10-methoxymethyl-, 9:10-bishydroxymethyl-, and 9:10-bisacetoxymethyl-1:2-benzanthracenes are all appreciably less active than 9:10-dimethyl-1:2-benzanthracene (Dunlap and Warren, 1946; Badger, Cook, Hewett, Kennaway, Kennaway, Martin and Robinson, 1940).

The type of substituent is clearly of great importance, but (as with methyl groups) the position of substitution is also of considerable importance, although it must not be assumed that the " favourable " positions for polar substituents are the same as the " favourable " positions for alkyl groups. Benzpyrene and 5-methyl-3:4-benzpyrene are potent carcinogens, for example, and 5-methoxy-3:4-benzpyrene is only very slightly active, while 8-methoxy-3:4-benzpyrene is a potent carcinogen (21st Annual Report, *B.E.C.C.*, 1944, p. 56).

The activity or otherwise of the metabolic products of polycyclic aromatic hydrocarbons is of some interest (see p. 332). 4′-Hydroxy-1:2-benzanthracene, the metabolic product of the non-carcinogenic 1:2-benzanthracene, is also inactive (23rd Annual Report, *B.E.C.C.*, 1946, p. 98). Similarly, 4′:8′-dihydroxy-1:2:5:6-dibenzanthracene, the metabolite of dibenzanthracene in mice and rats, is not carcinogenic. In rabbits 1:2:5:6 dibenzanthracene is metabolized to a dihydroxy dibenzanthracene of unknown orientation, and this product is also inactive as a carcinogen (Boyland, Levi, Mawson and Roe, 1941; Dobriner, Rhoads and Lavin, 1942). 8-Hydroxy-3:4-benzpyrene, one of the metabolites of benzpyrene, is only very slightly carcinogenic (21st Annual Report, *B.E.C.C.*, 1944, p. 56).

The above work mostly concerns the effect of substituents on carcinogenic hydrocarbons; but the effect of various groups on non-carcinogenic compounds is perhaps even more important. Benzanthracene is perhaps the most important non-carcinogenic hydrocarbon, as it is the parent substance of so many of the more interesting carcinogens. A large number of 10- substituted derivatives of benzanthracene has been examined, for substituents can easily be introduced into this position, either by substitution reactions, or by replacement. Many of these derivatives are tabulated in Table VII. Some derivatives are carcinogenic and some are inactive. In most cases the activity is of a low order, but the interesting feature of this work is that both electron-attracting and electron-repelling substituents can convert the inactive benzanthracene into a cancer-producing derivative. 10-Methyl-, 10-amino-, 10-mercapto-, and 10-methoxy-1:2-benzanthracenes are cancer-producing, but so also are the 10-cyano- and 10-aldehyde-derivatives. In this connection it is noteworthy that 9-methyl-10-cyano-1:2-benzanthracene is a potent carcinogen, with activity of the same order as that of 9:10-dimethyl-1:2-benzanthracene (Badger, Cook, Hewett, Kennaway, Kennaway, Martin and Robinson, 1940).

TABLE VII.—10-*Substituted*-1:2-*benzanthracenes.*

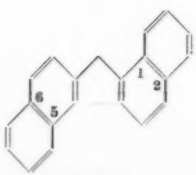
Substituent in the 10-position.	Carcinogenic activity. Skin.	References.	Subcutaneous tissue.	References.
$-CH_3$	+++	a	++++	b
$-CH_2CN$	..	..	+	d
$-CH_2Cl$	..	..	0	d
$-CH_2SH$	..	..	+	d, c
$-CH_2OH$	++	a	+++	d, a
$-CH_2OCH_3$	..	..	0	d
$-CH_2OCOCH_3$	++	a	+++	d, a
$-CH_2OCH_2CH_3$	+	a	0	a
$-CH_2NMe_2$	..	..	0	d
$-CH_2NEt_2$	..	..	0	d
$-CH_2COOH$	..	..	0	d
$-CH_2COOCH_3$	..	..	+	d
$-OH$	..	..	0	d
$-OCH_3$	..	..	++	d
$-NO_2$	..	..	0	d
$-NH_2$	..	..	+	d, a
$-CN$	+	e	++	d, a
$-SH$	..	..	+	e
$-NCO$	..	..	+	e
$-CHO$	..	..	++	d
$-CHOH.CH_3$	..	..	0	d
$-COCH_3$	0	e	..	..

a, Badger, Cock, Hewett, Kennaway, Kennaway, Martin and Robinson (1940); b, Shear (1938); c, Dunlap and Warren (1946); d, Shear, Leiter and Perrault (1940); e, Badger, Cook, Hewett, Kennaway, Kennaway and Martin (1942).

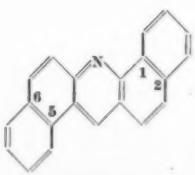
(d) *Heterocyclic and fluorene analogues.*

Following the discovery that 1:2:5:6-dibenzanthracene has moderate carcinogenic activity, it was of interest to examine certain heterocyclic analogues and related fluorene derivatives, and considerable work in this field has now been carried out. 1:2:5:6-Dibenzanthracene (XXI), 1:2:5:6-dibenzfluorene (XXII), 1:2:5:6-dibenzacridine (XXIII) and 1:2:5:6-dibenzcarbazole (XXIV) are all slightly to moderately active, while 1:2:5:6-dibenzphenazine (XXV) and *iso*-naphthathioxin (XXVI) appear to be inactive.

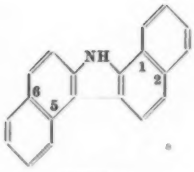
XXI.



XXII.



XXIII.

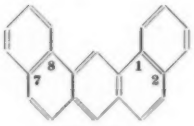


XXIV.

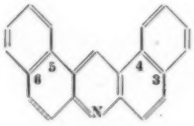
XXV.

XXVI.

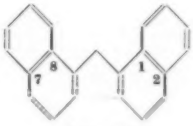
Again, 1:2:7:8-dibenzanthracene (XXVII), 3:4:5:6-dibenzacridine (XXVIII; 1:2:7:8-dibenzacridine in American numbering), 1:2:7:8:dibenzfluorene (XXIX), 1:2:7:8-dibenzcarbazole (XXX), and 3:4:5:6-dibenzcarbazole (XXXI) have given tumours, while 3:4:5:6-dibenzfluorene appears to be inactive (Hartwell, 1941). The effect of the introduction of one or more hetero atoms in the meso positions



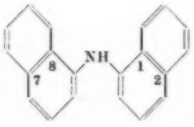
XXVII.



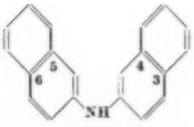
XXVIII.



XXIX.



XXX.



XXXI.

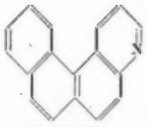
XXXII.

is therefore somewhat irregular, but many heterocyclic analogues may possess activity of a high order. 3:4:5:6-Dibenzcarbazole is particularly potent (Boyland and Brues, 1937; Andervont and Edwards, 1941). It is also of some interest that Kirby and Peacock (1946) have found *N*-methyl-3:4:5:6-dibenzcarbazole less effective than the parent substance in producing either tumours or hyperplastic liver changes in mice. 1:2-Benzcarbazole has been reported as very slightly carcinogenic by Schürch and Winterstein (1935), and this observation should clearly be confirmed by other workers. Several *N*-alkyl derivatives (methyl, ethyl, propyl) appear to be inactive (Buu-Hoï, 1946), so that *meso* substitution seems to be effective only in an aromatic hydrocarbon ring.

A few examples of derivatives with hetero atoms at positions other than the *meso* positions have been examined. 1′-Aza-3:4-benzpyrene (XXXIII) and 1′-aza-3:4-benzphenanthrene (XXXIV) appear to be completely inactive. 3-Azachrysene (XXXV), 5-aza-1:2-benzanthracene (XXXVI) and 4′-aza-1:2-benzanthracene (XXXVII), which are related to non-carcinogenic hydrocarbons, are also inactive (Badger, Cook, Hewett, Kennaway, Kennaway, Martin and Robinson, 1940; Shear and Leiter, 1941; Joseph, 1939). It is of some interest, however, that the latter compound, 4′-aza-1:2-benzanthracene, gave small tumour nodules

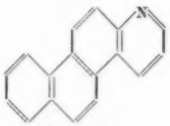
XXXIII.

1′-Aza-3:4-benzpyrene
3(N):4-Pyridinopyrene.



XXXIV.

1′-Aza-3:4-benzphenanthrene
Naphtho(1:2f)quinoline
3(N):4-Pyridinophenanthrene.

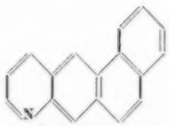


XXXV.

3-Azachrysene (British).
1-Azachrysene (American).
Naphtho(2:1f)quinoline.

XXXVII.

4′-Aza-1:2-benzanthracene
Naphtho(2:3f)quinoline
β-Anthraquinoline.

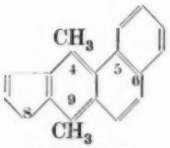


XXXVI.

5-Aza-1:2-benzanthracene
Naphtho(1:2g)quinoline.

in the kidney in 2 of 11 rats injected subcutaneously. 1 : 2-Benzanthracene itself appears to have some activity in rats, for it gave hepatomas in 2 of 6 rats of the Osborne and Mendel strain which were given this hydrocarbon by mouth (Sempronj and Morelli, 1939 ; White and Eschenbrenner, 1945).

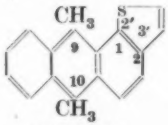
Activity of a very high order has been found in certain sulphur compounds closely related to 9:10-dimethyl-1:2-benzanthracene. 4:9-Dimethyl-5:6-benzthiophanthrene (XXXVIII) has produced tumours in an average time of 116



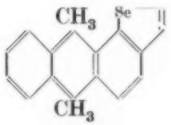
XXXVIII.

days, and thus has activity of the same order as that of the parent hydrocarbon (Dunlap and Warren, 1941 ; Sandin and Fieser, 1940). It also proved to be highly active when administered by injection ; tumours were produced in an

average of 18 weeks (100 per cent response), as compared with a latent period of 14 weeks with the hydrocarbon. Hershberg and Fieser (1941) have also prepared 9:10-dimethyl-1:2-(2′:3′-thiopheno)anthracene (XXXIX) and the corresponding selenium analogue (XL), but the biological tests do not seem to have been reported (Fieser, 1944).

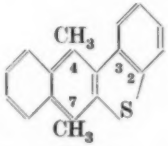


XXXIX.

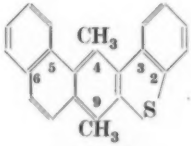


XL.

Compounds in which the sulphur atom replaces the phenanthrene-type double bond have been prepared by a group of workers under Sir Robert Robinson (23rd Annual Report, *B.E.C.C.*, 1946, p. 107). 4:7-Dimethyl-2:3:5:6-dibenzthionaphthene (XLI) was found to be moderately active by application to the skin, but proved inactive when administered by injection. On the other hand, 4:9-dimethyl-2:3:5:6-dibenzthiophanthrene (XLII), which again possesses a phenan-



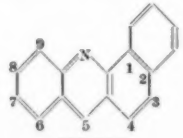
XLI.



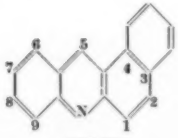
XLII.

threne-type double bond, is a particularly potent carcinogen both to the skin and to subcutaneous tissue. Furthermore, the isomeric 4:9-dimethyl-2:3:7:8-dibenzthiophanthrene is also a potent carcinogen (24th Annual Report, *B.E.C.C.*, 1947, p. 126).

The effect of alkyl substitution in the heterocyclic series of carcinogens has not been widely studied, except in the benzacridine series, and here some interesting differences between the 1:2-benzacridines and the 3:4-benzacridines have emerged. This work is particularly difficult to follow, as so many different systems of numbering the acridine molecule are in common use. The system used here is that commonly used by British chemists, but it differs from that used in America, where the Patterson system is used. It also differs from that currently used in French papers, where most of this work has been reported. Thus 3:4-benzacridine in Britain is the same as 1:2-benzacridine in America, and this is the same as 5:6-benzacridine in the French papers.

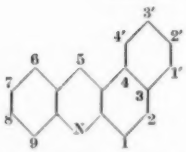


XLIII.
1:2-Benzacridine.

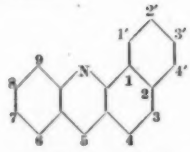


XLIV.
3:4-Benzacridine.

TABLE VIII.—*Methylbenzacridines.*



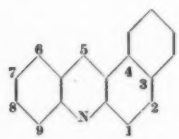
3:4-Benzacridine.*



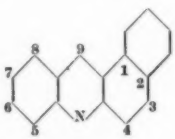
1:2-Benzacridine.

Compound.	Carcinogenic activity.			
	Skin.	References.	Subcutaneous tissue.	References.
3:4-Benzacridine	0	b	—	..
5-Methyl-	0	b	—	..
7-Methyl-	0	e	—	..
5:7-Dimethyl-	0	a	—	..
5:8-Dimethyl-	+	c, d	0	c
5:9-Dimethyl-	0	c, d	+	c
2′:5:9-Trimethyl-	—	..	+	c
5:7:9-Trimethyl-	+++	c	0	c
1:2-Benzacridine	0	b	—	..
5-Methyl-	+++	c	—	..
7-Methyl-	0	c	—	..
5:7-Dimethyl-	++++	c, d	+++	c
5:8-Dimethyl-	++++	c, d	+++	c
5:9-Dimethyl-	+++	c, d	+++	c
5:7:9-Trimethyl-	+++	c, d	++	c
5:6:7:9-Tetramethyl-	+++	c	+	c

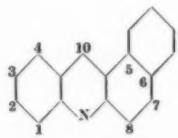
* Several different systems of numbering are in common use:



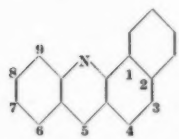
3:4-Benzacridine (in Britain).



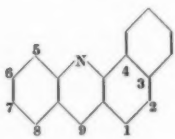
1:2-Benzacridine (in America).



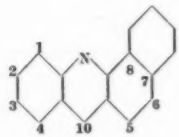
5:6-Benzacridine (in France).



1:2-Benzacridine (in Britain).



3:4-Benzacridine (in America).



7:8-Benzacridine (in France).

a, Quoted by Buu Hoï (1946), but method of test not stated; b, Quoted by Pullman (1947*a*), but method of test not stated; c, Lacassagne, Buu-Hoï, Lecocq and Rudali (1946); d, Lacassagne, Rudali, Buu-Hoï and Lecocq (1945); e, Bachmann, Cook, Dansi, de Worms, Haslewood, Hewett and Robinson (1937).

The mono-methyl derivatives of 3:4-benzacridine are either inactive, or only slightly active, but the introduction of several methyl groups, as in 5:7:9-trimethyl-3:4-benzacridine, gives rise to more potent derivatives. On the other hand, the mono-methyl derivatives of 1:2-benzacridine are, generally speaking, moderately or markedly carcinogenic. 5:9-Dimethyl-, 5:8-dimethyl-, and 5:7-dimethyl-1:2-benzacridines are extremely potent carcinogens, approaching methylcholanthrene in activity (Lacassagne, Rudali, Buu-Hoï and Lecocq, 1945; Lacassagne, Buu-Hoï, Lecocq and Rudali, 1946; Buu-Hoï, 1946).

(e) *Hydroaromatic compounds.*

The biological activity of hydrogenated derivatives of polycyclic aromatic hydrocarbons is of special interest, partly in view of the possibility that carcinogenic hydrocarbons may be formed *in vivo* from sterols or other normal constituents of animal cells, and partly with reference to the influence of the shape and size of the molecule on carcinogenic activity (Fieser, 1944).

In connection with the first point, it was clearly of importance to test dehydronorcholene (VII), the immediate precursor of methylcholanthrene in the laboratory preparation of this carcinogen from deoxycholic acid. It proved to be inactive, however (Shear, 1938; Bachmann, Cook, Dansi, de Worms, Haslewood, Hewett and Robinson, 1937).

It is also important that 6:7-dihydro-20-methylcholanthrene is inactive (Shear, Leiter and Perrault, 1941), for in this hydrocarbon only the phenanthrene-type double bond has been hydrogenated. On the other hand, hydrogenation does not always give rise to inactive compounds. 1′:2′:3′:4′-Tetrahydro-4:10-ace-1:2-benzanthracene is slightly active, having produced 4 tumours in 10 mice which received the compound by injection (Shear, 1938). No tumours were produced, however, with the related 1′:2′:3′:4′-tetrahydro-10-methyl-1:2-benzanthracene; nor with 9:10-dihydro-10-methyl-1:2-benzanthracene; nor with 5:6:7:8-tetrahydro-10-methyl-1:2-benzanthracene (Shear, 1939).

Of the compounds related to 3:4-benzpyrene which have been tested, 1′:2′-dihydro-4′-methyl-3:4-benzpyrene proved to have about the same activity as the corresponding fully aromatic compound, but 1′:2′:3′:4′-tetrahydro-3:4-benzpyrene gave no tumours when injected into mice, although the duration of the experiment was 20 months (Shear, 1939).

THE METABOLISM OF POLYCYCLIC AROMATIC HYDROCARBONS

So far as is known the carcinogenic and related non-carcinogenic polycyclic aromatic hydrocarbons all undergo hydroxylation or perhydroxylation in the animal body, and there does not seem to be any essential difference between the metabolism of the carcinogens as opposed to the non-carcinogens. There is some species specificity, however, in that rabbits metabolize the hydrocarbons by a process which differs slightly from that of mice and rats (Boyland and Weigert, 1947).

α-Naphtholglycuronic acid has been isolated from the urine of dogs dosed with naphthalene, and l-α-naphthylmercapturic acid from the urine of rabbits. Recently Young (1946) has isolated l-1:2-dihydroxy-1:2-dihydronaphthalene from the urine of rats dosed subcutaneously or orally with naphthalene, and following

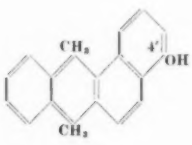
similar experiments with rabbits Booth and Boyland (1947) have obtained a dl-1:2-dihydrodiol.

Anthracene is metabolized by a similar process. The major product in the urine of both rats and rabbits is a 1:2-dihydrodiol and monoglycuronide. Rabbits appear to produce dl-1:2-dihydroxy-1:2-dihydroanthracene, and rats the corresponding l-compound (Boyland and Levi, 1935 ; 1936*a*, *b*).

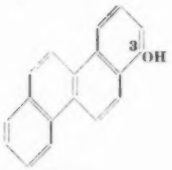
Such dihydrodiols are very readily dehydrated to phenols, and it is, therefore, not necessarily significant that dihydrodiols have not been isolated following experiments with the more complex hydrocarbons. In such experiments only phenols have been isolated (or characterized by the formation of a more stable methyl ether), although there is some evidence for the formation of such dihydrodiols (Weigert and Mottram, 1946*a*, *b*).

Berenblum and Schoental (1943*b*) have found that 1:2-benzanthracene is metabolized to the 4′-hydroxy-derivative (XLV), and the potent cancer-producing substance 9:10-dimethyl-1:2-benzanthracene is probably metabolized to 4′-hydroxy-9:10-dimethyl-1:2-benzanthracene (XLVI) (22nd Annual Report, *B.E.C.C.*, 1945, p. 53). In both cases rats were used, and no experiments with rabbits have yet been reported. Chrysene is metabolized to 3-hydroxychrysene (XLVII ; 1-hydroxychrysene in American numbering) (Berenblum and Schoental, 1945). Similarly, 3:4-benzpyrene is metabolized to 8-hydroxy-3:4-benzpyrene (XLVIII), although some 10-hydroxy-3:4-benzpyrene is also formed in small quantity. In rabbits the process is similar except that more of the 10-hydroxy- derivative is formed (Berenblum and Schoental, 1946*b*).

XLV.



XLVI.



XLVII.

XLVIII.

1:2:5:6-Dibenzanthracene is oxidized to 4′:8′-dihydroxy-1:2:5:6-dibenzanthracene in rats and mice (Dobriner, Rhoads and Lavin, 1942), but to another dihydroxy derivative (of unknown orientation) in rabbits (Boyland, Levi, Mawson and Roe, 1941 ; Dobriner, Rhoads and Lavin, 1942 ; Badger, 1947).

These metabolism experiments are of considerable interest for two reasons. Firstly, as Boyland and Weigert (1947) have emphasized, the species differences in metabolism may not be unconnected with carcinogenic action. Although 1:2:5:6-dibenzanthracene readily produces tumours at the site of injection in rats and in mice, it very rarely, if ever, produces such tumours in rabbits. Secondly, it is noteworthy that in rats the polycyclic aromatic hydrocarbons all give rise to phenols in which the hydroxyl groups occupy comparable positions. The 4′-position of benzanthracene (and of its 9:10-dimethyl- derivative) is comparable with the 3- position in chrysene, and with the 8- position in 3:4-benzpyrene. In general, however, oxidizing reagents do not attack these positions (Cook and Schoental, 1947), and the biological oxidation must therefore be conditioned by additional or other factors. It must be admitted, however, that in all metabolic experiments the end-product isolated represents only a small fraction of the

amount of hydrocarbon administered. The greater part of the hydrocarbon must be broken down into simple products which escape isolation.

THE CHEMICAL REACTIVITY OF CARCINOGENS.

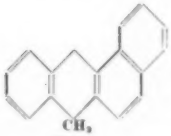
Many of the most potent carcinogenic hydrocarbons are extremely reactive, and take part in substitution and addition reactions with very great facility. This pronounced chemical reactivity of some, but not all the carcinogens has attracted the attention of several workers, and many attempts have been made to find correlations between the chemical properties of the molecules and their cancer-producing activity. Probably the most extensive work in this field has been carried out by L. F. Fieser and his associates, who, in a number of papers, have developed the hypothesis that carcinogenicity may be associated with some specific chemical reaction (Fieser, 1938 ; 1944).

The addition of hydrogen to polycyclic aromatic hydrocarbons and to the cancer-producing substances in particular is complex. With phenanthrene there are three pronounced stages of hydrogenation, leading to 9:10-dihydro-, to 1:2:3:4-tetrahydro-, and 1:2:3:4:5:6:7:8-octahydrophenanthrene. Similarly, with anthracene there are three stages of reduction. Mild reduction gives the 9:10-dihydro- derivative, and more vigorous treatment gives the 1:2:3:4-tetrahydro- and 1:2:3:4:5:6:7:8-octahydro- derivatives. Most of the carcinogens are derivatives of phenanthrene, and many are also derivatives of anthracene. The hydrogenation of such compounds may take a complex course, involving either the anthracene moiety, the phenanthrene moiety, or both.

The reduction of 1:2-benzanthracene has been investigated by Fieser and Hershberg (1937). With sodium and amyl alcohol a hexahydride (XLIX) was obtained ; but with hydrogen and platinum 5:6:7:8-tetrahydro-1:2-benzanthracene (L) was formed. Reduction of the cancer-producing 10-methyl-1:2-benzanthracene proceeded in a similar fashion ; sodium and amyl alcohol gave the corresponding hexahydride, and hydrogen and platinum the corresponding tetrahydride. Furthermore, treatment of 10-methyl-1:2-benzanthracene with sodium, followed by treatment with alcohol, gave the dihydride (LI) expected from an anthracene derivative. These authors also made the very interesting observation

XLIX.

L.

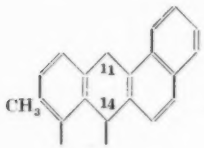


LI.

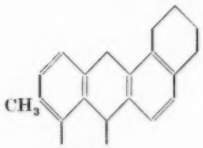
that the *meso* dihydride (LI) on further hydrogenation with hydrogen and platinum gave the hexahydride (corresponding to XLIX), which shows conclusively that the tetrahydrides (L) are not produced *via* the *meso* dihydrides, as might be expected.

The reduction of methylcholanthrene is again related both to the anthracene and to the phenanthrene types. Bachmann (1936) obtained the 11:14-dihydride (LII) by the action of sodium, followed by alcohol, on the parent hydrocarbon. On the other hand, Fieser and Hershberg (1938*a*) found that reduction of methyl-

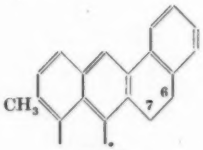
cholanthrene with sodium and amyl alcohol gave the hexahydride (LIII), and the same hexahydride was also obtained by reduction with hydrogen and platinum, together with some 6:7-dihydro-20-methylcholanthrene (LIV).



LII.

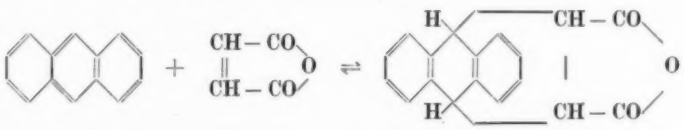


LIII.



LIV.

The *meso* positions in anthracene derivatives are always reactive, and it was therefore of some interest to determine whether carcinogenicity is in any way bound up with this reactivity. As early as 1931, however, Cook (1931) found that the carcinogenic 1:2:5:6-dibenzanthracene is less reactive than anthracene in addition reactions involving the *meso* carbon atoms. Anthracene and its derivatives undergo a Diels-Alder type of addition with maleic anhydride to form 9:10-*endo*succinic anhydride derivatives:



Bachmann and Kloetzel (1938) found that in organic solvents the reaction is reversible, and that with different anthracene derivatives the velocity of the addition varies enormously. The presence of methyl groups in the *meso* positions was found greatly to facilitate the reaction. 9-Methylanthracene reacted faster than anthracene, and 9:10-dimethylanthracene reacted faster still. Indeed, with the latter hydrocarbon the addition proceeded at room temperature. The presence of phenyl groups in the *meso* positions was found to have the opposite effect, markedly retarding the reaction. Bachmann and Kloetzel's work indicated that there is no correlation between reactivity of this nature and carcinogenic activity, for they found that methylcholanthrene reacts about as fast as benzanthracene, and 1:2:5:6-dibenzanthracene was found to react only slowly.

The photo-oxidation of carcinogenic and related compounds is another addition reaction involving the *meso* carbon atoms which has been investigated. Anthracene itself forms a photo-oxide (Dufraise and Gérard, 1935, 1936, 1937) (LV), and certain *meso* substituted anthracene and benzanthracene derivatives, and

LV.

even more especially certain naphthacene and pentacene derivatives, form photo-oxides with very great facility. Shabad (1945) has noted that when 9:10-dimethyl-1:2-benzanthracene is exposed to light and air for 2 or 3 months it showed

an appreciable decrease in carcinogenic activity. Similar observations were made by Bradbury, Bachmann and Lewisohn (1941), who found that the pure hydrocarbon in acetone protected from light and air produced tumours in an average of 2·0 months, while the corresponding time for the same hydrocarbon in acetone *not* protected from light and air was found to be 4·7 months. These observations are clearly associated with photo-oxidation. Several cancer-producing hydrocarbons such as 9:10-dimethyl-1:2-benzanthracene readily form photo-oxides, but this property is not an invariable characteristic of the carcinogens. Benzpyrene, for example, gave no photo-oxide (Cook and Martin, 1940 ; Cook, Martin and Roe, 1939).

Clar (1941) has emphasized that the linear benz-homologues of anthracene, such as naphthacene, pentacene, hexacene, and heptacene, undergo all transannular addition reactions, such as photo-oxidation and the addition of maleic anhydride with increasing facility. Naphthacene and pentacene are known to be non-carcinogenic, and it is abundantly clear therefore that *meso* reactivity is not associated with carcinogenic activity. Additional evidence to this effect has been provided by Iball (1940 ; Fieser and Dietz, 1931), who studied the oxidation-reduction potentials of the quinones derived from carcinogenic and related non-carcinogenic hydrocarbons. The relative values of the potentials obtained under identical conditions gives a quantitative comparison of the chemical reactivity associated with the 9:10-positions of the nucleus. Iball was unable to find any correlation between the observed potential and the carcinogenic activity of the hydrocarbon.

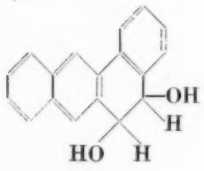
The coupling of diazonium reagents with various hydrocarbons has been investigated by Fieser and Campbell (1938), and many of the most potent carcinogens, including 3:4-benzpyrene, methylcholanthrene and cholanthrene were found to react or couple very readily. With these compounds an intense coloration was produced in a few minutes. These observations are interesting, for this reaction was previously thought to be confined to phenols, amines, enols and, to a much smaller extent, ethylenic compounds, especially conjugated dienes. On the other hand, there is no specificity in the reaction for 10-methyl-1:2-benzanthracene, 5:10-dimethyl-1:2-benzanthracene and other potent carcinogens did not react, and several non-carcinogenic hydrocarbons reacted readily.

There have been many attempts to study the oxidation of polycyclic aromatic hydrocarbons with special reference to carcinogenesis. Most oxidizing agents react with anthracene and its derivatives to give *para* quinones. Phenanthrene and its derivatives are normally oxidized to *ortho* quinones. Compounds in which both systems are present, as in benzanthracene, normally react predominantly as derivatives of anthracene. Chromic acid, for example, oxidizes benzanthracene to 1:2-benzanthra-9:10-quinone. Although chromic acid oxidation of 1:2:5:6-dibenzanthracene gives rise to 1:2:5:6-dibenzanthra-9:10-quinone, some 1:2:5:6-dibenzanthra-3:4-quinone is also formed (Cook, 1933*a*).

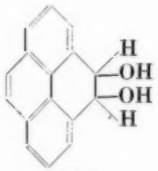
The effect of special or unusual oxidizing reagents has been investigated with interesting results. Warren (1943), for example, found that certain hydrocarbons undergo aerobic oxidation in the presence of ascorbic acid, and that this oxidation is inhibited by KCN and by H_3PO_3, but is unaffected by hydrogen peroxide. Anthracene is oxidized to anthraquinone, and benzpyrene to a mixture of 3:4-benzpyrene-5:8-quinone and the 5:10-quinone, together with an alkali-soluble compound which could not be isolated. This reaction is of special interest,

for Kennaway, Kennaway and Warren (1944) have found that benzpyrene and some other carcinogens caused an increase in the concentration of ascorbic acid in the livers of mice, while naphthalene, anthracene and phenanthrene caused no such increase.

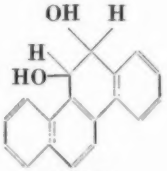
Cook and Schoental (1947) have investigated the oxidation of polycyclic aromatic hydrocarbons with osmium tetroxide. In the presence of pyridine this reagent was found to add exclusively to adjacent carbon atoms to form complexes which, on hydrolysis, gave rise to dihydrodiols. Indeed, osmium tetroxide appears to be a "double bond" reagent in that it does not (under these conditions) attack reactive centres, but only very reactive aromatic double bonds, and ethylenic double bonds. 1:2-Benzanthracene was oxidized to the 3:4-dihydrodiol (LVI), pyrene to the 1:2-dihydrodiol (LVII) and chrysene to the 1:2-dihydrodiol (LVIII). Related carcinogenic hydrocarbons, such as 9:10-dimethyl-1:2-benz-



LVI.



LVII.



LVIII.

anthracene, methylcholanthrene, and benzpyrene also reacted to give similar diols.

In spite of the fact that this reagent attacks double bonds, it will be noted that the position of attack differs from that experienced in biological oxidation (p. 332). If benzanthracene undergoes perhydroxylation *in vivo*, as is commonly assumed in order to account for the isolation of phenolic derivatives, this biological oxidation must take place at the 3′:4′-bond, and yet osmium tetroxide attacks the 3:4-bond. Again, anthracene is oxidized *in vivo* to the 1:2-dihydrodiol, a compound which contains an ethylenic double bond. With osmium tetroxide, anthracene gives the 1:2:3:4-tetrol (Cook and Schoental, 1948).

Perbenzoic acid is another reagent which probably attacks aromatic double bonds in preference to reactive centres. Eckhardt (1940*a*) has studied the rate of oxidation of several compounds with this reagent. The oxidation products were not determined, the oxidation simply being followed iodometrically over a 7–15-day period. Certain carcinogens such as methylcholanthrene and benzpyrene reacted very rapidly, while other non-carcinogens, such as pyrene, reacted only slowly. The correlation was not complete, however, and the reaction should certainly be further investigated.

The oxidation of polycyclic aromatic hydrocarbons with lead tetra-acetate has been investigated by Fieser and Hershberg (1938*c*). Here, again, some of the carcinogens reacted with great facility, while others proved to be more resistant to oxidation. Furthermore, some non-carcinogens were attacked while some carcinogens were unaffected. 1:2-Benzanthracene was converted into the 10-acetoxy- derivative, but the carcinogenic 1:2:5:6-dibenzanthracene was not attacked. It has been suggested that this lack of reactivity in *meso* positions adjacent to an angular ring is due to steric effects, but there is some reason to believe that the

9- position in benzanthracene and the 9:10 positions in dibenzanthracene are inherently less reactive than the *meso* positions in anthracene (Iball, 1940; Pullman, 1947*a*). Other carcinogenic agents, such as 10-methyl-1:2-benzanthracene, methylcholanthrene, and benzpyrene, were readily attacked by lead tetra-acetate. Benzpyrene gave 5-acetoxy-3:4-benzpyrene (Fieser and Hershberg, 1939), a product which is analogous to that obtained from benzanthracene. 10-Methyl-1:2-benzanthracene was attacked on the methyl group to give 10-acetoxymethyl-1:2-benzanthracene, and methylcholanthrene was attacked on the methylene group directly attached to the *meso* position. The reaction, therefore, provides a useful method for probing for the active centres in a molecule, whether such centres are located in the nucleus or in a side chain.

Badger and Cook (1939) extended the reaction to the very feebly active 9:10-dimethylanthracene, and the potent carcinogen, 9:10-dimethyl-1:2-benzanthracene, and found that both hydrocarbons react very readily. The methyl groups were found to be attacked in each case, the products isolated being 9:10-di(acetoxymethyl)anthracene and 9:10-di(acetoxymethyl)-1:2-benzanthracene. High reactivity of this nature is, therefore, not associated with the cancer-producing properties of the molecule. More recently Fieser and Putman (1947) have made an extensive survey of the effect of lead tetra-acetate on hydrocarbons. They concluded that although some correlation between chemical reactivity and carcinogenic potency is apparent, this correlation is not perfect. The rate of reaction of the 10-alkyl-1:2-benzanthracenes remained practically the same, although cancer-producing activity falls off rapidly as the length of chain increases. Furthermore, the feebly active 9:10-dimethylanthracene was actually found to react faster than the potent carcinogen 9:10-dimethyl-1:2-benzanthracene. And finally, the moderately carcinogenic 1:2:5:6-dibenzanthracene does not react with lead tetra-acetate.

Further evidence as to the reactivity of *meso* methyl groups was provided by Badger and Cook (1939), who brominated 9:10-dimethyl-1:2-benzanthracene. Both methyl groups were attacked, the product being 9:10-bisbromomethyl-1:2-benzanthracene. Barnett and Matthews (1926) had previously observed the same sort of reactivity in 9:10-dimethylanthracene, which they brominated to 9:10-bisbromomethylanthracene. It is of some interest, therefore, that Badger and Cook (1940) found that 9-methyl-1:2-benzanthracene and 6-methyl-1:2-benzanthracene are not brominated in the methyl group, but are substituted in the nucleus to give the corresponding 10-bromo- derivatives. The same paper also records the facile preparation of 10-bromo-1:2-benzanthracene from the parent hydrocarbon. It is also noteworthy in this connection that *meso*-acetyl-1:2-benzanthracene is converted into *meso*-trichloroacetyl-1:2-benzanthracene by treatment with sodium hypochlorite (Dansi and Ferri, 1939).

Many other substitution reactions have been investigated. Benzanthracene has been chloromethylated to give 10-chloromethyl-1:2-benzanthracene (Badger and Cook, 1939). 1:2-Benzanthranyl-10-mercaptan has been prepared by allowing benzanthracene to react with sulphur monochloride followed by sodium sulphide (Wood and Fieser, 1940). Benzanthracene has been nitrated, the major product being 10-nitro-1:2-benzanthracene (Barnett and Matthews, 1925; Fieser and Hershberg, 1938*c*). Benzanthracene reacts with methylformanilide to give the 10-aldehyde (Fieser and Hartwell, 1938). It has also been thiocyanated to give both 10-thiocyano-1:2-benzanthracene (57 per cent) and 9-thiocyano-1:2-

benzanthracene (5 per cent) (Wood and Fieser, 1941). Benzanthracene has also been subjected to a variety of Friedel-Crafts reactions. With ethylchloroglyoxalate, in nitrobenzene, 1:2-benzanthranyl-10-glyoxylic acid was produced in good yield (Badger and Cook, 1940). With acetyl chloride in nitrobenzene a *meso* acetyl derivative (almost certainly the 10-acetyl-) was produced (Dansi and Ferri, 1939). With acetic anhydride, however, the reaction was found to be rather more complex and the products isolated were: *meso*-acetyl-, 7-acetyl-, 6-acetyl-, and two acetyl derivatives of unknown orientation (Cook and Hewett, 1933). With oxalyl chloride both the 10-carboxylic acid and 4:10-oxalyl-1:2-benzanthracene were obtained (Dansi, 1937). Further, by heating benzanthracene and chloroacetic ester with copper at a high temperature Dansi and Ferri (1939) obtained some ethyl 1:2-benzanthranyl-10-acetate.

Relatively little work has been carried out on the direct substitution of carcinogens other than that already mentioned, and no satisfactory chemical distinction between carcinogenic and non-carcinogenic hydrocarbons has yet been discovered. 3:4-Benzpyrene, for example, is very readily thiocyanated to the 5-thiocyano- derivative; but anthracene also reacts readily, to give 9:10-dithiocyanoanthracene (Wood and Fieser, 1941), and there is clearly no specificity in the reaction. The chemical work with this reagent is interesting, however. 10-Methyl-1:2-benzanthracene was found to be substituted in the free 9- position, to give 9-thiocyano-10-methyl-1:2-benzanthracene; and 9-methyl-1:2-benzanthracene was found to be substituted in the free 10- position to give the 10-thiocyano- derivative. On the other hand, methylcholanthrene was attacked in the methylene group directly attached to the *meso* position. It is useful to compare the actions of lead tetra-acetate, and of bromine, on these hydrocarbons so far as the work has been carried out.

A few interesting substitution reactions have been carried out with benzpyrene. It is readily chlorinated in the 5- position (Windaus and Raichle, 1939), and it is nitrated to 5-nitro-3:4-benzpyrene (Windaus and Rennhak, 1937; Fieser and Hershberg, 1939; Eckhardt, 1940*b*). Windaus and Rennhak have also described a number of other substitution products, including a tribromo-derivative and a sulphonic acid. It is interesting that acetylation of benzpyrene gives mainly the 10-acetyl-derivative (Windaus and Raichle, 1939; Fieser and Hershberg, 1939). Benzpyrene also reacts readily with methylformanilide to give the 5-aldehyde (Fieser and Hershberg, 1938*c*). This reagent reacts more readily with anthracene than with benzanthracene, and the carcinogenic 1:2:5:6-dibenzanthracene is not attacked even under considerably more drastic conditions.

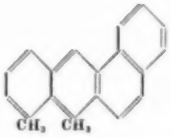
THE INFLUENCE OF THE SHAPE AND SIZE OF THE MOLECULE.

It has often been suggested that carcinogenic activity is associated with certain optimum molecular dimensions, and that the shape and size of the molecule is of paramount importance for the development of carcinogenic activity. As early as 1935, for example, Barry, Cook, Haslewood, Hewett, Hieger and Kennaway remarked that their results were "in keeping with the view that there is an optimum state of molecular complexity for carcinogenic activity." The hypothesis was not, at first, described at length, but it was clearly the basis for much of the synthetic work.

Much attention has been paid to the "simplification" of the molecules of known carcinogens by the replacement of benzene rings with 5-membered rings

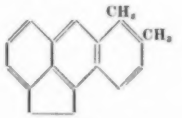
or by one or more alkyl groups. This process led, for example, from 1:2:5:6-dibenzanthracene to 5:6-*cyclo*penteno-1:2-benzanthracene, and to 5:6-dimethyl-1:2-benzanthracene, to 5-methyl- and to 6-methyl-1:2-benzanthracene. All these compounds were found to be moderately active. In the same way cholanthrene and methylcholanthrene have been "simplified." 5:10-Dimethyl-1:2-benzanthracene (LX) is an extremely active carcinogen, as is cholanthrene (LIX). 1:2-*cyclo*Penteno-5:10-aceanthrene (LXI) is slightly to moderately active. Further "simplification" to 1:2-dimethyl-5:10-aceanthrene (LXII) to 1:2-*cyclo*penteno-5-methylanthracene (LXIII), and to 5:10-aceanthrene (LXIV), however, gives rise to inactive compounds (Shear, Leiter and Perrault, 1941 ; Shear, 1938).

LIX.



LX.

LXI.



LXII.

LXIII.

LXIV.

Many examples of this type of "simplification" could be quoted, and it was this which led Hewett (1940) to point out that nearly all the carcinogens are derivatives of phenanthrene substituted in two or more of the positions 1, 2, 3 and 4. Further substitution of 2:3-benzphenanthrene (i.e. 1:2-benzanthracene), for example, in either or both of the remaining 1- and 4- positions gives cholanthrene, 3:4-benzpyrene, and 9:10-dimethyl-1:2-benzanthracene, all of which are strongly carcinogenic. Again, further substitution of 3:4-benzphenanthrene in the remaining 1- or 2- positions also leads to pronounced activity, as in 1:2:3:4-dibenzphenanthrene, 1-methyl-3:4-benzphenanthrene, and 2-methyl-3:4-benzphenanthrene. Again, further substitution of 1:2-benzphenanthrene (i.e. chrysene) in either or both of the remaining 3- or 4- positions also leads to carcinogens, notably 1:2-dimethylchrysene. It is significant that 1:2:3:4-tetramethylphenanthrene is also slightly carcinogenic.

The hypothesis that the shape and size of the molecule is of paramount importance was stated at length by Bergmann (1942). He suggested (i) that the molecule of a carcinogen acts as a whole, and that the shape and size of the molecule determine its activity ; (ii) that all carcinogens may be conceived as parts of an "ideal" carcinogenic structure ; and (iii) that every substance which imitates more or less closely a given parent structure resembles it also in its cancer-producing action. In what amounts to an extension of the hypothesis, Lettré (1944) pointed out that when the polycyclic aromatic hydrocarbons are considered as derivatives of anthracene and phenanthrene, those which still possess a plane of symmetry through the centre of the middle ring of the two original

hydrocarbons are never carcinogenic. According to this author the loss of this symmetry by substitution is a necessary but not sufficient condition for carcinogenic activity.

This hypothesis that carcinogenic activity is determined by optimum molecular dimensions is of considerable interest, and it has several advantages over any other hypothesis which has so far been announced. On the other hand, it is by no means completely satisfactory. The heterocyclic analogues and the fluorene analogues of the carcinogenic hydrocarbons provide considerable support for the hypothesis, but also many exceptions. It is of considerable interest that the methyl derivatives of 1:2-benzacridine (3:4-benzacridine in American numbering, and 7:8-benzacridine in the French papers) are very active carcinogens, as might be predicted by analogy with the corresponding benzanthracenes. On the other hand, the corresponding 3:4-benzacridines (1:2-benzacridines in American numbering, and 5:6-benzacridines in the French papers) are either inactive or have only slight activity. Again, 4:9-dimethyl-5:6-benzthiophanthrene (XXXVIII) has almost the same activity as the corresponding hydrocarbon, 9:10-dimethyl-1:2-benzanthracene, either when tested by painting on the skin or by subcutaneous injection. Yet 4:7-dimethyl-2:3:5:6-dibenzthionaphthene (XLI) is only moderately active to the skin of mice, and inactive when administered by injection.

Often quoted in support of the hypothesis that molecular architecture is of predominant importance in regard to carcinogenic activity is the observation of Dodds, Lawson and Williams (1941) that α-ethyl-β-*sec*-butylstilbene has produced cancers in mice. This hydrocarbon (LXV) is, of course, an " open model " of 3:4-benzpyrene. Too much weight should not be given to this isolated example,

LXV.

however, for ethyl*sec*butylstilbene produced only 2 tumours in 100 mice, and in view of this very low order of carcinogenicity and to the known impossibility of avoiding occasional contamination (Earle, 1943 ; Hieger, 1946 ; Anderson, 1947), the significance of the observation must remain in some question. Benzanthracene itself has produced 1 tumour in 80 mice, and this hydrocarbon is almost invariably quoted as being non-carcinogenic.

In spite of much evidence which tends to support the view that the shape and size of the molecule governs the activity, it is difficult to believe that molecular architecture *alone* is the governing factor. It is noteworthy that activity is to be found in compounds as simple as 9:10-dimethylanthracene and 1:2:3:4-tetramethylphenanthrene and as large and complex as 1:2:3:4-dibenzpyrene and 3:4:8:9-dibenzpyrene, while many compounds of intermediate complexity are completely devoid of activity.

Some interesting experiments by Lacassagne and his co-workers may have some significance in this connection (Lacassagne, Buu-Hoï and Cagniant, 1944 ; Lacassagne, Buu-Hoï and Rudali, 1945 ; Lacassagne, Buu-Hoï, Daudel and Rudali, 1944). These workers made repeated application to the skin of mice with solu-

tions containing a mixture of two hydrocarbons of similar molecular configuration, but one of which is weakly carcinogenic (or inactive) and the other strongly carcinogenic. The pairs of compounds were: 1:2:5:6-dibenzacridine and 1:2:5:6-dibenzanthracene; chrysene and methylcholanthrene; 1:2:5:6-dibenzfluorene and methylcholanthrene. Tumours were produced more slowly in each case than when the potent hydrocarbon alone, at the same concentration, was applied. Lacassagne supposes that the two substances, having the same affinity because of their analogy of structure, penetrate into the same cells, where they are fixed in the same substrate. Each molecule of the weakly active substance, by fixing itself in the cell, hinders the fixation of the molecule of potent carcinogen, and thus delays the appearance of tumours. The interesting feature of this work is that although the weakly active substances can hinder the rapid production of tumours, presumably by "blocking" the "receptors," they still lack the total requirements for rapid carcinogenic activity. It seems likely, therefore, that some feature in addition to the optimum molecular dimensions is necessary for the development of potent carcinogenic activity.

THE IMPORTANCE OF THE K POSITION.

It has long been recognized that most of the carcinogens of the polycyclic aromatic type are derivatives of phenanthrene, although all derivatives of phenanthrene are not carcinogenic. In an attempt to determine whether the phenanthrene ring system is essential, 9:10-dimethylanthracene and 1:2:3:4-tetramethyl phenanthrene were submitted to extensive tests (Badger, Cook, Hewett, Kennaway, Kennaway and Martin, 1942; Kennaway, Kennaway and Warren, 1942). Both compounds are closely related to the potent carcinogen 9:10-dimethyl-1:2-benzanthracene. Both compounds were found to be slightly active, and it can only be concluded therefore that while phenanthrene is a common component of carcinogens of this type it is not absolutely essential.

In spite of this result, evidence has continued to accumulate that the phenanthrene ring system is of special significance. Robinson (1946) has suggested that an activated phenanthrene-type bridge is implicated in most carinogens, and there is considerable evidence in favour of this hypothesis. The hydrogenation of the phenanthrene-type bridge in methylcholanthrene, for example, gives 6:7-dihydro-20-methylcholanthrene, which is devoid of activity; although it must be admitted that this result is inconclusive in view of the somewhat uncertain effect of hydrogenation on carcinogenic activity (p. 331). The results obtained with certain sulphur analogues of 9:10-dimethyl-1:2-benzanthracene (p. 329) are also inconclusive, but also provide some support for the hypothesis. Again, it is of considerable interest that although 1:2:3:4-dibenzphenanthrene is a potent carcinogen, the 9- and 10- methyl derivatives of this compound are inactive (Badger, Cook, Hewett, Kennaway, Kennaway, Martin and Robinson, 1940; Harris and Bradsher, 1946). This suggests that the phenanthrene double bond must be unsubstituted, and that the presence of the methyl group offers some sort of "interference." It is, perhaps, unfortunate that these results have not been confirmed by the skin-painting technique as well as by subcutaneous injection. In any case, a similar relationship does not seem to hold with the 3- and 4-methyl-1:2-benzanthracenes, which are both moderately active by injection, although only feebly so by application to the skin. Furthermore, 7-methyl-1:2:3:4-

dibenzpyrene, like the parent hydrocarbon, is a potent carcinogen, and the phenanthrene double bond is again substituted.

In a further approach to the problem, Pullman (1947*a*, *b*, *c*, *d*), and Pullman and Pullman (1946*a*, *b*), have developed a theory relating carcinogenic activity with an optimum density of π electrons on the phenanthrene-type double bond, or the K position as it has been called. A method for the calculation of such electronic charges has been worked out. This method is based on the resonance theory by which benzene is considered to be a *hybrid* of the structures :

A. B. C. D. E.

Summing the electronic charges required for each of these structures and calculating that the Dewar forms (C, D and E) contribute 22 per cent to the benzene hybrid and the Kekulé forms (A and B) the remaining 78 per cent, Pullman was able to draw a molecular diagram showing the distribution of the π electrons. The calculations for tetracyclic molecules are complex, but many of these structures have now been worked out. Methyl groups act as slight donors of electrons to a ring system, and it is the essence of the Pullman theory that this increase in electronic charge converts an inactive structure (such as benzanthracene) into a carcinogen. Pullman has calculated the electronic charge of the K position for many methyl derivatives of benzanthracene, of benzphenanthrene, of 1:2-benzacridine and of 3:4-benzacridine (Table IX). The effect of a methyl group on the K position was found to vary considerably, depending on the position of the methyl group. The *meso* positions in benzanthracene, for example, were found to have the most pronounced effect, and the effect of more than one methyl group is additive. The critical charge on the double bond, below which the compounds are not carcinogenic, was found to be about $1{\cdot}29e$. At first sight the correlation between the electronic charge and the carcinogenic activity is extremely good—in fact rather *too* good, considering the uncertain nature of the approximations required in the theoretical treatment. When set out as in Table IX, however, where all the compounds have been arranged in order of increasing charge, together with assessments of activity towards skin of mice and to subcutaneous tissue, certain irregularities become apparent; the benzphenanthrenes are especially evident, for example. Furthermore, the evidence for the *upper* limit to the optimum electronic charge is rather slender.

Clar (1941) has examined the absorption spectra of many polycyclic aromatic hydrocarbons, and has deduced interesting relationships between structure, reactivity and the wave lengths of the absorption bands. He associates certain bands with the " Dewar " forms of the hydrocarbons, and other bands with the " Kekulé " forms. Some evidence is presented that the shift in wave length (towards longer wave length) by the linear or angular addition of benzene rings, etc., can be correlated with the colour of the hydrocarbon and its " reactivity." If this correlation is valid it should be possible to compare the wave length of the appropriate absorption band with the electronic charge as obtained by Pullman, and with the carcinogenic activity. In benzanthracene, for example, Clar associates the absorption bands of the Kekulé form with the bond which

Pullman calls the K position. It is of interest, therefore, that Jones (1940, 1943) has found a limited correlation between the wave length of this absorption band and the carcinogenic activity. In the limited series of the methylbenzanthracenes the correlation is quite good; but the correlation cannot be extended to other hydrocarbons, and, indeed, breaks down with the alkylbenzanthracenes other than the methyl derivatives. In this connection it may be mentioned that there is no correlation to be found between the wave length of the fluorescence bands and carcinogenic activity. Nor is there any correlation between carcinogenic activity and the intensity of fluorescence (Bruce, 1941; Bruce and Todd, 1939; Hieger, 1930; Berenblum and Schoental, 1946*a*).

In view of the very extensive calculations which would be required Pullman did not calculate the electronic charge for representative pentacyclic hydrocarbons; but on general grounds these compounds were considered to support the theory. It is not difficult, for example, to understand—on the basis of this theory—why 1:2:3:4-dibenzanthracene is not carcinogenic, why 1:2:7:8-dibenzanthracene is only very feebly carcinogenic, and 1:2:5:6-dibenzanthracene is moderately active. Furthermore, the theory also provides a reasonable explanation for the relatively feeble activity of 9:10-dimethyl-1:2:5:6-dibenzanthracene; it is only necessary to assume that the electronic charge is *above* the optimum by virtue of the two *meso* methyl groups in addition to the benz- rings. On the other hand, 9-methyl-1:2:5:6-dibenzanthracene must have a somewhat lower charge (nearer the optimum) and is strongly carcinogenic. Similarly, it is also understandable why 9:10-dimethyl-1:2:7:8-dibenzanthracene is strongly carcinogenic and 9:10-dimethyl-1:2:3:4-dibenzanthracene is inactive. Again, the effect of the *meso* nitrogen atom, as in 1:2:5:6-dibenzacridine, may be to reduce the charge on the K position and hence the carcinogenic activity. The effect of two nitrogen atoms, as in 1:2:5:6-dibenzphenazine, will be greater, and this compound is inactive.

In a further paper Pullman (1947*c*) has attempted to explain the observation that while 5:6-*cyclo*penteno-1:2-benzanthracene is a moderately active carcinogen, 6:7-*cyclo*penteno-1:2-benzanthracene is only feebly active. It was suggested that by virtue of the Mills-Nixon effect the two structures (LXVI) and (LXVII) predominate for these compounds, owing to the " bond fixation " imposed by the

LXVI.

LXVII.

five membered rings. For this reason Pullman suggests that the charge on the K region is very much greater in the case of the 5:6 compound than in the 6:7 derivative. On the other hand, it must be remembered that 5:6-dimethyl-1:2-benzanthracene is potent, and that 6:7-dimethyl-1:2-benzanthracene is only very slightly active (Barry, Cook, Haslewood, Hewett, Hieger and Kennaway, 1935). Furthermore, 1:2:5:6-dibenzanthracene is a moderate carcinogen, while 1:2:6:7-dibenzanthracene is inactive (Barry *et al.*, 1935). It is therefore possible that the effect is due as much to the position of substitution as to any " bond fixation."

In any case, the 6:7 derivative has many other structures which allow for the Mills-Nixon effect than that represented in (LXVII).

A further examination of the Pullman theory reveals many important exceptions. One would expect the methylbenzanthracenes with methyl groups in the angular ring to be active, yet all these compounds are inactive or have only trace activity. The inactive compounds of this type (all of which must be considered as exceptions) include 1′-methyl-, 2′-methyl-, 3′-methyl-, 4′-methyl-, 1′:10-dimethyl-, 2′:6-dimethyl-, 2′:7-dimethyl-, 3′:6-dimethyl- and 3′:7-dimethyl-1:2-benzanthracenes. It is also surprising that acenaphthanthracene is only slightly active, and that 3:9-dimethyl- and 5:8-dimethyl-1:2-benzanthracenes are inactive and that 7-methyl-8:9-ace-1:2-benzanthracene is inactive.

Again, as has been detailed in p. 324, a study of 10-substituted 1:2-benzanthracenes has shown conclusively that both electron-attracting and electron-repelling groups can convert an inactive parent hydrocarbon, such as benzanthracene, into a cancer-producing derivative. It is interesting to compare the effect of a methyl group with that of the cyano group, for example. 10-Methyl-1:2-benzanthracene and 9:10-dimethyl-1:2-benzanthracene are potent carcinogens. 10-Cyano-1:2-benzanthracene is only slightly active, but 9-methyl-10-cyano-1:2-benzanthracene is a particularly potent compound.

In spite of these exceptions, the Pullman theory has much to commend it as a basis for further work. The charge of π electrons is directly associated with "reactivity," and Badger (1948) has therefore attempted to examine the validity of the calculations by an experimental method. Osmium tetroxide is known to add to the K position of many carcinogenic and related compounds (Cook and Schoental, 1947), and a method for measuring the rate of addition has been devised (Badger, 1948; Badger and Reed, 1948). Using this method the rate of addition of osmium tetroxide to 18 carcinogenic and related non-carcinogenic hydrocarbons and derivatives has been studied. Most of these compounds were derivatives of benzanthracene, but other representative carcinogens were also included. Other things being equal, osmium tetroxide should add more rapidly to those compounds with the greater charge on the K position. In agreement with Pullman it was found that methyl groups, especially in "favourable" positions, have a pronounced effect on the rate of reaction, and hence, presumably, on the charge at the K position. 9:10-Dimethyl-1:2-benzanthracene and other *meso* substituted benzanthracenes were found to react very much faster than benzanthracene itself, although compounds with methyl groups substituted at positions far from the K position showed only a very slight increase in reactivity. On the other hand, osmium tetroxide was found to react rapidly with both acenaphthanthracene and with 2′:7-dimethyl-1:2-benzanthracene. Indeed, the very slightly carcinogenic acenaphthanthracene reacted at about the same rate as cholanthrene—a very potent carcinogen. Furthermore, 2′:7-dimethyl-1:2-benzanthracene, which is inactive, reacted at about the same rate as 1:2-dimethylchrysene, which is a moderately potent carcinogen. These two compounds, acenaphthanthracene and 2′:7-dimethyl-1:2-benzanthracene, are, however, also exceptions to the Pullman theory, and the agreement is therefore quite good. It was also found, however, that derivatives of 3:4-benzphenanthrene reacted very much slower than benzanthracene, which is unexpected from the figures quoted in support of the theory by Pullman. Furthermore, phenanthrene itself reacted very much slower than benzanthracene, which is not the

TABLE IX.—*Density of π Electrons at K Position and Carcinogenic Activity.*

Compound.	Density of π electrons at K position (Pullman). e.	Carcinogenic activity.	
		Skin.	Subcutaneous tissue.
Naphthacene	1·258	0	—
Anthracene	1·259	0	0
Triphenylene	1·260	0	—
3:4-Benzacridine	1·260	0	—
1:2-Benzacridine	1·270	0	—
Chrysene	1·272	0	0
5-Methyl-3:4-benzacridine	1·273	0	—
Naphthalene	1·274	0	—
1:2-Benzanthracene	1·283	0	0
5:8-Dimethyl-3:4-benzacridine	1·284	+	0
5:7-Dimethyl-3:4-benzacridine	1·285	0	—
5:9-Dimethyl-3:4-benzacridine	1·286	0	+
Phenanthrene	1·291	0	—
8-Methyl-1:2-benzanthracene	1·292	+	0
5-Methyl-1:2-benzacridine	1·293	+++	—
3:4-Benzphenanthrene	1·293 (1·293)	+	0
7-Methyl-1:2-benzanthracene	1·294	+	+
6-Methyl-1:2-benzanthracene	1·294	+	—
9-Methyl-1:2-benzanthracene	1·296	++	+++
5-Methyl-1:2-benzanthracene	1·296	++	++
3-Methyl-1:2-benzanthracene	1·298	+	++
4-Methyl-1:2-benzanthracene	1·298	+	++
5:7:9-Trimethyl-3:4-benzacridine	1·298	+++	0
5:9-Dimethyl-1:2-benzacridine	1·302	+++	+++
5:8-Dimethyl-1:2-benzacridine	1·304	++++	+++
5:7-Dimethyl-1:2-benzacridine	1·304	++++	+++
10-Methyl-1:2-benzanthracene	1·306	+++	++++
5:6-Dimethyl-1:2-benzanthracene	1·307	+++	—
5:9-Dimethyl-1:2-benzanthracene	1·309	—	++++
8-Methyl-3:4-benzphenanthrene	1·309 (1·305)	+	0
6-Methyl-3:4-benzphenanthrene	1·310 (1·302)	+	+
4:9-Dimethyl-1:2-benzanthracene	1·311	—	+++
1-Methyl-3:4-benzphenanthrene	1·312 (1·308)	++	0
2-Methyl-3:4-benzphenanthrene	1·312 (1·308)	+++	+
5:7:9-Trimethyl-1:2-benzacridine	1·312	+++	++
7-Methyl-3:4-benzphenanthrene	1·313 (1·304)	+	0
5:10-Dimethyl-1:2-benzanthracene	1·317	—	++++
9:10-Dimethyl-1:2-benzanthracene	1·319	++++	+++
4:10-Dimethyl-1:2-benzanthracene	1·321	—	++
6:9:10-Trimethyl-benzanthracene	1·330	++++	++
5:9:10-Trimethyl-benzanthracene	1·332	++++	+++
5:6:9:10-Tetramethylbenzanthracene	1·343	+++	+

result expected from the Pullman calculations. Recently, however, Berthier, Coulson, Greenwood and Pullman (1948) have calculated the bond orders for the tetracyclic aromatic hydrocarbons by the method of molecular orbitals. These calculations are in better agreement with the kinetic experiments with osmium tetroxide in that 3:4-benzphenanthrene is given a lower bond order than benzanthracene ; but this is an observation which casts further doubt on the validity of the correlation as observed by Pullman.

It is also noteworthy that Badger found that 10-cyano-1:2-benzanthracene reacted very much slower than benzanthracene, and that the potent carcinogen 9-methyl-10-cyano-1:2-benzanthracene reacted slightly slower than the inactive benzanthracene. This is in agreement with general electronic theory, but not with the Pullman theory relating carcinogenic activity with the electronic charge on the K position.

It is of some interest to compare these results with those of Eckhardt (1940*a*), who examined the rate of oxidation of several polycyclic derivatives with perbenzoic acid. The site of oxidation is not known, but it is probably the K position. Eckhardt observed an increase in the rate of reaction following the introduction of a methyl group, and a decrease following the introduction of deactivating groups, such as —CHO and $-NO_2$. Here again, however, no complete correlation between reactivity and carcinogenic activity was observed.

It can only be concluded, therefore, that no single theory yet advanced satisfactorily accounts for the carcinogenic activity of all the polycyclic aromatic hydrocarbons and their derivatives. The hypothesis that the shape and size of the molecule governs the activity seems partly true, but will not account for many observations. Similarly, the Pullman theory, while accounting almost quantitatively for the effect of methyl groups on inactive parent hydrocarbons, fails to account for the many exceptions. It is possible that there is an element of truth in both theories, and this may be supported by the fact that the exceptions to the one theory seem to be moderately well " explained " by the other. It may be that the carcinogenic hydrocarbons interact with some tissue component to form a " complex," and that the ease of formation and the stability of this complex is governed by (*a*) the molecular dimensions of the molecule, *and* (*b*) the charge of π electrons at one or more of the double bonds, particularly the K position. The shape and size of the molecule can be conceived as assisting in the formation of the complex by providing the best " fit " with the specific cellular receptors. In view of the pronounced aromatic character of all the carcinogens of the polycyclic type, it seems likely that the forces which bind the hydrocarbon to the tissue component are the same as those which bind these substances to polynitro compounds, to antimony pentachloride, stannic chloride, etc. It must not be expected, however, that there is any correlation between carcinogenic activity and ability (or even enhanced ability) to form complexes with such simple components. It must be admitted, however, that the introduction of a methyl group enhances the stability of the resulting picrate, and also deepens its colour. Furthermore, deactivating groups depress the stability of picrates, and such complexes as are formed are mostly lighter in colour. Most of the potent carcinogens form very dark red or purple picrates, a circumstance which is clearly related to their aromatic character, but which is not necessarily correlated with ability to produce cancers. Unfortunately, very little is known of these forces (Weiss, 1942). In any case, the complete elucidation of the

problem of the relationship between chemical structure and carcinogenic activity must be left to further investigation.

SUMMARY.

1. Certain tricyclic, tetracyclic, pentacyclic and hexacyclic aromatic hydrocarbons or their homologues are cancer-producing. Most of the active compounds, but not all, are derivatives of phenanthrene.

2. Many closely related heterocyclic compounds are also cancer-producing. So are certain dibenzfluorenes.

3. The introduction of methyl groups into benzanthracene, benzphenanthrene and chrysene normally leads to enhanced activity. Two methyl groups, or more, normally act additively. On the other hand, there seems to be a limit beyond which the introduction of further methyl groups does not lead to enhanced activity. The introduction of methyl groups into the angular ring of benzanthracene does not have this effect, and all such compounds are either inactive or have only trace activity.

4. Similar relationships hold for the benzacridines, except that the 1:2-benzacridines are potentially much more carcinogenic than the 3:4-benzacridines.

5. The introduction of methyl groups into benzpyrene and dibenzanthracene is more complex. In many cases the effect is to diminish the activity, although examples of the opposite effect are also known.

6. Alkyl groups other than methyl are progressively less effective as the number of carbon atoms in the chain increases.

7. Many other substituents in " favourable " positions can convert an inactive parent hydrocarbon into a cancer-producing derivative. Both electron-attracting and electron-repelling substituents may have this property. The " favourable " positions for methyl substitution are not necessarily the same as the " favourable" positions for other substituents.

8. Partial hydrogenation of a carcinogenic compound sometimes, but not always, deactivates the substance.

9. The carcinogenic substances are—so far as is known—active as such and are metabolized to inactive derivatives.

10. The carcinogenic and related non-carcinogenic hydrocarbons are metabolized by the same mechanism, namely, hydroxylation or perhydroxylation. This oxidation takes place at centres other than those normally attacked by chemical oxidizing agents.

11. There is no correlation between chemical " reactivity " and carcinogenic activity.

12. Attempts to relate carcinogenic activity (*a*) with the shape and size of the molecule, and (*b*) with the electronic charge on the phenanthrene-type double bond present in most carcinogens, have been only partly successful.

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CLINICAL TRIALS OF TETRA-SODIUM 2-METHYL-1 : 4-NAPHTHOHYDROQUINONE DIPHOSPHATE, IN CONJUNCTION WITH X-RAY THERAPY.

J. S. MITCHELL,

From the Radiotherapeutic Centre, Addenbrooke's Hospital and the Department of Radiotherapeutics, University of Cambridge.

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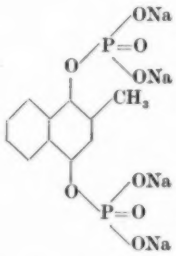
THIS paper is an account of clinical trials of the use of a chemical agent, tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate, to attempt to improve the results of the radio-therapy of cancer. This compound is already in use in medicine as a synthetic vitamin-K substitute, and is of very low toxicity even in the large doses employed.



Tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate.

This work has been in progress since November, 1946, and 116 cases have been treated. So far, it has been limited almost entirely to the palliative treatment of patients with malignant tumours, usually very advanced, which were not likely to respond satisfactorily to radiotherapy. Examples of most of the common types of cancer have been studied. For carcinoma of the bronchus, an attempt has been made to assess, in a relatively small series of histologically confirmed cases, the possible improvement by the chemical agent of the palliative results of X-ray therapy.

This approach to the problem of attempting to improve the treatment of cancer by the combined use of radiotherapy and chemotherapeutic agents was suggested by the finding that therapeutic doses of radiation inhibit the synthesis of thymonucleic acid in growing cells, including those of malignant tumours (Mitchell, 1942, 1943, 1944, 1946; Euler and Hevesy, 1942; Ahlström, Euler and Hevesy, 1944; Hevesy, 1945; Stowell, 1945; Holmes, 1947). The aim is to choose a chemical agent to block the synthesis of the nucleic acids by a mechanism different from that of X and gamma radiation. An obvious limitation of this method of approach is that probably the most important mechanism by which radiations kill cells is the production of gross structural changes in the chromosomes following chromosome breakage, reunion and interchange, and

this is not directly dependent upon the inhibition of synthesis of nucleic acids. Mitotic inhibition *per se* appears to be less important.

The compound was selected on the basis of attempting to block a reaction of the type described by Kalckar (1945) by means of a phosphoric ester containing a stable condensed aromatic nucleus of much the same molecular dimensions as a purine and with oxidation-reduction properties after hydrolysis. It is emphasized that this line of reasoning may not be correct.

Experiments with tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate have been reported previously (Mitchell and Simon-Reuss, 1947). It was then shown that this compound produces mitotic inhibition in chick fibroblast cultures and in some human carcinomata. In the tissue cultures, significant potentiation of the effects of X-radiation and of the compound in inhibiting mitosis was found under suitable conditions.

The anti-mitotic action of quinones including 2-methyl-1 : 4-naphthoquinone and the corresponding hydroquinone, is well known and has been described by Lehmann (1942) and Lehmann, Lüscher and Huber (1945) in the case of Tubifex eggs, and by Meier and Allgöwer (1945), who worked with chick fibroblast cultures. Badger, Elson, Haddow, Hewett and Robinson (1942) found no significant inhibition of the growth of the Walker rat carcinoma 257 by 1 : 4 naphthoquinone, 2-methyl-1 : 4-naphthoquinone or the corresponding hydroquinone diacetate. However, Euler and Solodkowska (1947) found retrogression of the Jensen rat sarcoma after subcutaneous injection of naphthoquinone. As far as I am aware, no clinical trials of these or related compounds have been previously reported.

The laboratory investigations, including histological studies, in connection with the present work will be described elsewhere. An analysis of the anti-mitotic activity of certain quinones has been published by Friedmann, Marrian and Simon-Reuss (1948) of this Department. Tissue culture methods have been used for the sorting and testing of compounds of possible interest acting alone and in combination with small doses of X-radiation. The importance of interaction of anti-mitotic agents with —SH compounds has been emphasized although it is likely that this is not the only mechanism involved. It is of interest that 1 : 4-naphthohydroquinone bis-hydrogen succinate has been found to be entirely inactive as a mitotic inhibitor in the tissue cultures, in striking contrast to the diphosphate. The rôle of the phosphate group which appears to be important, perhaps especially in the case of intravenous administration, is uncertain.

Dosage and methods of administration.

Tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate, $6H_2O$ (molecular weight 530·22) is a water-soluble synthetic vitamin K substitute. The concentration of the solution used clinically refers to the anhydrous salt. This will be referred to as "the compound." The preparation used was "Synkavit," manufactured and kindly given by Roche Products Limited.

The most frequently used unit dose was 100 mg. of compound which was supplied in 2 c.c. of solution in ampoules for intramuscular and intravenous injection.

This order of dose was suggested by the tissue culture experiments (Mitchell and Simon-Reuss, 1947), from which it appeared that a concentration of at least 3×10^{-6} M. was required for appreciable potentiation of the inhibition of mitosis

produced by small doses of X-radiation. This dose was of course much greater than that used for vitamin K activity, for which the manufacturers supply ampoules containing 10 mg. of the compound in 1 c.c. of solution, but its safety was strongly suggested by published information on the toxicity of the compound in animals. The acute LD50 for subcutaneous injection was found to be 450 mg. per kg. for mice, 675 mg. per kg. for chicks (Foster, 1940) and 610 mg. per kg. for rats (Smith, Ivy and Foster, 1943). Chronic administration of 100 mg. per kg. daily for 18 days produced no pathological effect in rats ; in rabbits, a temporary fall in red blood cell count was produced at this dose level, with more serious changes at higher doses (Smith, Ivy and Foster, 1943). Similar findings have also been reported for related compounds (Molitor and Robinson, 1940 ; Fromherz, 1941). Mrs. B. E. Holmes of this Department also kindly carried out confirmatory experiments on the toxicity in rats of the preparation of the compound used.

After cautious clinical testing, a level for safe dosage and a scheme of administration was found. For most of the cases reported in this paper, the compound was administered by intramuscular injection. Since February, 1948, intravenous administration has been used and appears to be more effective. Oral administration has been used but is of uncertain value.

The general scheme of administration is to give a course of daily injections, starting 3 to 7 days before the beginning of the X-ray treatment and continuing throughout the course of X-ray treatment.

It is probably wise to start *intravenous injections* with a test dose of 10 mg. For most patients, the best basic daily dose for intravenous injection is probably 100 mg. Larger intravenous doses can, and probably should, be given ; by cautiously increasing the daily dose in steps of 100 mg., the largest single dose administered has been 700 mg. but the maximum tolerated daily intravenous dose appears to be about 300 mg. The largest total doses administered in a course of intravenous injections have been 7310 mg., given in 19 injections in an overall time of 26 days, and 8140 mg. given in 31 injections on consecutive days. These very large doses have been used in some cases without X-rays to attempt to produce a carcinostatic effect. At these high levels, it is necessary to decide on the individual doses from day to day and occasionally to interrupt the course of injections.

The compound is not irritant and never has produced venous thrombosis at the site of the injection.

For intramuscular injection, the unit daily dose used most often has been 100 mg. and this has been continued in many cases for as long as 6 weeks, with no attributable toxic effects. However, much larger doses can be given with safety. For example, one patient received 500 mg. intramuscularly for 42 days, and another 1000 mg. intramuscularly daily for 11 days, without any demonstrable toxic effects, and with only occasional faintness and nausea.

Prolonged oral administration has been attempted, e.g. one patient took 100 mg. daily (in tablets) for 114 consecutive days, without any toxic effect during the following 14 months.

Toxic effects and side actions.

The freedom from toxicity of the compound, even in the highest doses used, is striking. None of the toxic effects observed in animals at dosage levels higher

by a factor of at least 10 and often 50 have been encountered in patients. In particular, no significant changes in the red cell count, haemoglobin, or total or differential white cell count have been found ; nor has there been any evidence of renal damage, or of changes in colour of the hair.

There appears to be no risk of producing thrombosis. Among the 116 cases treated with the compound, there has been one instance of a coronary thrombosis, but this was almost certainly not attributable to the compound.

An unexpected effect of large intravenous doses of the compound is the production of focal pain in the tumour in some cases, mainly those in which the tumour is involving bone, as in advanced cancer of the mouth. The pain in the region of the tumour, and referred from it, often starts almost immediately during the intravenous injection which is generally given in the usual way into the median cubital vein. The pain may last for an hour and in several cases, when doses of 400 mg. and higher have been given, has been sufficiently severe to require morphia and of course subsequent reduction of the dose. Usually, however, an analgesic such as two tablets of Veganin given beforehand is sufficient to control the pain. In only one case, that of a woman aged 76 years with an advanced squamous carcinoma of the floor of the mouth involving the mandible and fungating at the chin, has pain been observed in the region of the tumour after an intravenous dose of 100 mg.

The production of pain in the tumour is the factor limiting the dose in some cases, but more often nausea, vomiting and faintness, and sometimes a feeling of illness prevent the use of the highest doses. Transient nausea sometimes appears shortly after an intravenous dose of 200 mg. and occasionally vomiting after the first injection of 300 mg. in a course, but there is great individual variation. Transient right-sided upper abdominal discomfort has been observed in several cases.

The cause of the pain in the region of the tumour, especially when bone is involved, is probably cell oedema, of the type observed in the tissue cultures as an effect of the compound.

It is of interest that apparently similar focal pain in tumours is produced by another chemical agent, the polysaccharide of *Chr. prodigiosum* (Shear, 1947 ; Holloman, 1947).

With intramuscular injection, there are negligible side effects. Focal pain has never been observed and only occasionally after high doses such as 500 mg. have faintness and nausea occurred.

On the question of possible deleterious effects of large doses of the compound on the reproductive organs, it is suggested that, while so far there has been no evidence of damage, it is wise in general to avoid the use of the compound in patients in the reproductive period of life, and never to use it in children.

Outline of Investigation.

This work started with a general survey of the effects of the compound used in conjunction with palliative X-ray therapy in advanced, and often very advanced cases of most of the commoner types of cancer. It appeared that a small degree of improvement of the results of palliative X-ray therapy was obtained especially in some cases of advanced carcinoma of the mouth, larynx and pharynx, carcinoma of the breast with pulmonary metastases and carcinoma of the bronchus.

The difficulties of assessment of the results are obvious. However, in the case of carcinoma of the bronchus where the results of even radical X-ray therapy are so poor, an attempt has been made to study the influence of the ancillary use of the compound on the survival time after X-ray therapy. A small series of histologically verified cases of inoperable carcinoma of the bronchus treated by X-ray therapy and the compound in May to December, 1947, have been examined, together with a control group of comparable cases selected with the same criteria and treated in the same department as far as possible by the same methods of X-ray therapy alone from 1944 to May, 1947. The difficulties of this method are recognized but the use of alternate cases as controls was attempted and found impractical. It is difficult to exclude a systematic error, but if it is assumed that this has not occurred, the results of the combined use of the compound and X-ray therapy show a small but significant improvement upon the palliative results of X-ray therapy alone in carcinoma of the bronchus.

In the work mentioned so far, the compound has been administered by intramuscular injection. Since February, 1948, an investigation has been in progress to try to determine the value of intravenous administration of the compound when used in conjunct on with both palliative and radical X-ray therapy and also when used alone. In general, it appears that the results with intravenous administration of the compound are perhaps somewhat better than those with intramuscular injection.

DISCUSSION OF RESULTS.

General survey.

The general survey of the possible therapeutic effects of the compound, when administered by intramuscular injection, in combination with X-ray therapy, includes 73 patients with various types of advanced malignant tumours, other than carcinoma of the bronchus. Of these 73 patients, 37 are dead and 36 still living (on 20.viii.48). An unexpectedly good palliative response was observed in at least 10 out of the 37 dead, and at least 13 out of the 36 living.

Of the 37 dead, the 10 cases responding unexpectedly well were : 2 cases of very advanced carcinoma of the mouth, surviving 4 and 11 months ; 1 case of extensive bilateral secondary cervical glands from a previous carcinoma of the lip, surviving 12 months ; 1 case of extrinsic carcinoma of the larynx involving the right pyriform fossa with fixed secondary glands, surviving 1 year 7 months ; 2 cases of advanced post-cricoid carcinoma, both surviving 8 months ; and 4 cases of cancer of the breast, 2 with advanced secondary supraclavicular glands surviving 8 and 11 months, and 2 with pulmonary metastases surviving 7 and 10 months. The 27 cases showing no improvement of the response were all very advanced and included : carcinoma of breast, 4 cases ; carcinoma of ovary, 4 ; carcinoma of mouth, including tongue, 4 ; extrinsic carcinoma of larynx, 1 ; post cricoid carcinoma, 1 case (surviving 4 months) ; carcinoma of antrum, 1 case ; carcinoma of stomach, 1 ; carcinoma of oesophagus, 2 ; carcinoma of colon, 2 ; carcinoma of rectum, 1 ; teratoma testis, 1 ; reticulosarcoma, 2 ; lymphosarcoma, 1 ; squamous carcinoma of skin, 1 ; and melanoma, 1.

Of the 36 cases still living, the 13 showing unexpectedly good palliative response were : carcinoma of the tongue, 4 cases, of which 3 were in the posterior third ; recurrent anaplastic carcinoma of the nasal fossa, 1 ; extrinsic carcinoma

of larynx involving the pyriform fossa with fixed secondary glands, 2; post-cricoid carcinoma with pulmonary metastases, 1; carcinoma involving the whole breast with mobile axillary glands, 1; osteosarcoma of ilium, 1; and carcinoma of the skin 3 cases, of whom 2 were of extremely extensive basal cell lesions of the face with gross tissue destruction, which had been radioresistant but appeared to become radiosensitive with the combination of the compound and X-radiation. The 23 cases showing no improvement of the response were: carcinoma of mouth including tongue, 4; secondary glands from carcinoma of lip, 2; intrinsic carcinoma of larynx, 1; carcinoma of oesophagus, 2; inoperable carcinoma of colon, 1; squamous carcinoma of anus, 1; carcinoma of vulva, 1; squamous carcinoma of skin, 1; fungating carcinoma of breast, 1; carcinoma of uterine cervix, stage IV, 2 cases; advanced ovarian carcinoma with ascites, 2 cases; advanced carcinoma of bladder, 1; mediastinal tumour, 1; and cerebral tumour, 3 cases.

Of especial interest are the 2 cases of advanced basal cell carcinoma of the face which were radioresistant after much previous treatment and apparently became radiosensitive again with the combination of the compound and radical X-radiation.

It is important to emphasize that the use of the compound does not prevent the development of metastases, or obviously reduce their incidence.

Carcinoma of the bronchus.

The first criterion for the selection of the cases is histological verification of carcinoma of the bronchus either at bronchial biopsy or post-mortem.

The *control* group, treated by X-radiation alone, consists of 13 cases, with the following times of survival in months, to the nearest month, from the beginning of the X-ray treatment: 8, 8, 7, 6, 5, 4, 4, 3, 2, 2, 2, 2, 2. Of these, 4 received radical X-ray therapy, with minimum tumour doses of 5000 r. in 5 weeks, 4500 r. in 25 days, 3250 r. in 23 days, and 3000 r. in 18 days; these cases survived 8, 4, 6, and 8 months respectively. The remaining 9 cases received palliative X-ray therapy, with smaller doses and usually larger fields. The histological verification was at post mortem in 4 cases, which survived 8, 5, 2 and 2 months; the diagnosis in the remaining 9 cases was based on bronchial biopsy. All the cases in the control group are dead.

The *treated* group, which received X-ray therapy and the compound by intramuscular injection, consists of 9 cases, with the following times of survival in months, to the nearest month, after the beginning of treatment: for the 2 cases still living (with symptoms) on 20.ix.48, 12 and 11, and for those dead, 9, 9, 9, 6, 5, 4, 3. All received palliative X-ray therapy except the one case which survived 4 months and had been given a minimum tumour dose of 3000 r. in 20 days. All but one case surviving 9 months were diagnosed on the bronchial biopsy.

Although the groups are small, the results of the combined use of the compound and X-ray therapy show a small improvement in length of survival, which must be regarded as significant, upon the results of X-ray therapy alone. Assuming that the parent distributions from which the control and treated groups are drawn, are not grossly different from normal, as discussed below, it appears valid to apply Student's t test. The absence of systematic error must be assumed.

For the control and treated groups specified above, the mean survival time is 4·23 months for the *control* group and 7·56 months for the *treated* group. The difference between these mean survival times is significant, since for 20 degrees of freedom, $t = 2{\cdot}838$ and $P_F = 0{\cdot}010$.

For the members of the control and treated groups receiving palliative X-ray therapy, the mean survival time is 3·22 months for the controls and 8·00 months for the treated group. The difference between these two sub-groups is very significant; for 15 degrees of freedom, $t = 3{\cdot}98$[3] and $P_F = 0{\cdot}002$.

The mean survival time in the *control* group, treated by X-ray therapy alone, is in agreement with the results of other workers. Schinz and Zuppinger (1937) reported that for 69 cases, the mean survival time after the beginning of X-ray treatment was 4·4 months; 16 cases (23 per cent) lived more than 6 months and 4 cases more than 1 year. Dobbie (1944) found that of 111 cases receiving palliative X-ray therapy, 26 per cent lived more than 6 months, and of 48 cases treated by radical X-ray therapy 54 per cent lived more than 6 months.

In our own cases, treated by X-ray therapy alone in 1944–47, inclusion of histologically unverified cases for which the clinical diagnosis was reasonably certain and which survived 2 months or more, gives a group of 47 cases with a mean time of survival after the beginning of treatment of 5·23 months. This group is comparable with the whole control group specified above and has a distribution which is not normal, although the divergence from normality is not very convincing ($P = 0{\cdot}03$[1]). Inclusion of all the cases with a reasonably certain diagnosis treated in this department before May, 1947, by X-ray therapy alone gives a group of 79 cases with a mean life of 4·15 months. None of these are still living; 12 cases lived more than 6 months and only one case lived more than 1 year (13 months).

The consistently bad results obtained in carcinoma of the bronchus after X-ray therapy alone with comparable techniques used in different centres makes it possible to carry out an investigation such as that described with reasonable certainty that the results are not invalidated by systematic error.

The results described showing a small increase in the length of survival after X-ray therapy due to the ancillary use of the compound are in accordance with clinical impressions. The improvement in the palliative effect was striking in 3 of the 9 cases in the treated group. Of these 3 cases, one was a woman of 41 years of age who was extremely ill when admitted, and returned temporarily to almost normal health but died after 9 months; the other two were men of 59 and 60 years of age, and were still living on 20.xi.48, though with returning symptoms due to recurrence, after 13 and 14 months. The unexpectedly good immediate palliative response in these 3 cases was not obviously correlated with the histology but was associated with an unusually severe skin reaction to the radiation. In this connection it is of interest to note that the rare cases responding anomalously well to X-ray therapy alone often show an unusually severe skin reaction.

The use of the compound did not prevent the development of metastases, including cerebral metastases, which occurred in the one of the 2 female patients in the treated group, who survived 6 months.

It is concluded that intra-muscular administration of the compound produces a small but useful improvement in the palliative results of X-ray therapy in some cases of inoperable carcinoma of the bronchus.

Intravenous administration.

Administration of the compound by intravenous injection has been used in 20 cases. Of these, 6 cases were in combination with radical X-ray therapy and included : advanced carcinoma of mouth, 2; carcinoma of bronchus, 2; post-cricoid carcinoma, 1; and adeno-carcinoma of ethmoid, 1 case. The 4 cases in combination with palliative X-ray therapy were : advanced carcinoma of mouth, 1; advanced carcinoma of colon fungating through anterior abdominal wall, 1; and carcinoma of ovary, 2. The 10 cases in which the compound has been used alone had all received previous radical X-ray or radium treatment and had recurrences for which in the usual way no further treatment could be done. These cases were : carcinoma of tongue with widespread extension and fixed secondary glands, 2; secondary adenocarcinoma in lymph glands of left supra-clavicular region and posterior triangle, with primary in transverse colon, 1; carcinoma of bronchus, 2; extrinsic carcinoma of larynx with fixed secondary glands, 1; fibrosarcoma of neck, 1; secondary glands from carcinoma of ear, 1; melanoma with hepatic and pulmonary metastases, 1; and generalized reticulo-sarcoma, 1. In at least 4 cases there was subjective improvement including relief of pain. In the 2 cases of advanced squamous carcinoma of the tongue and the case of secondary adenocarcinoma in glands of neck, in addition to the subjective improvement, there was some retrogression of the tumours. This was confirmed histologically in the case of the secondary adenocarcinoma. It is emphasized that these effects are only palliative and require much further investigation.

SUMMARY.

Clinical trials of large doses of tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate, a synthetic vitamin K substitute, mainly in conjunction with palliative X-ray therapy, have been carried out in 116 patients with advanced malignant tumours. The compound is of very low toxicity and is administered by intramuscular and perhaps preferably, intravenous injection. Large intravenous doses produce focal pain in the region of the tumour in some cases, especially where there is bone involvement.

A general survey of the use of the compound, administered by intramuscular injection, in combination with X-ray therapy, showed an unexpectedly good palliative response in at least 23 out of 73 patients with various common types of advanced malignant tumours, other than carcinoma of the bronchus.

In a small series of histologically verified cases of inoperable carcinoma of the bronchus, the treated group of 9 cases who received X-ray therapy and the compound by intramuscular injection, showed a mean time of survival after the beginning of X-ray treatment of $7{\cdot}5^{6}$ months, while the control group of 13 cases, receiving only X-ray therapy, had a mean survival time of $4{\cdot}2^{3}$ months. For the members of the treated and control groups who received palliative X-ray therapy, the corresponding mean survival times were $8{\cdot}0^{0}$ months and $3{\cdot}2^{2}$ months. These differences between the treated and control groups are considered to be significant.

Intravenous administration of the compound has been used in 20 cases. It was used alone in 10 very advanced cases and in 3 of these there was at least subjective improvement.

It is concluded that tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate in large doses produces a small but useful improvement in the palliative results of X-ray therapy in some cases of advanced cancer.

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POLAROGRAPHIC ANALYSIS OF NORMAL AND PATHOLOGICAL SERA.

ALICE M. ROBINSON.

*From the Pathology Department, St. Bartholomew's Hospital, London, E.C.*1.

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BRDICKA (1933) discovered that sulphur-containing proteins gave characteristic polarographic waves when examined in ammonia buffer solutions containing traces of cobalt salts. Application of this method of analysis to normal and carcinomatous sera showed that the latter had smaller quantities of sulphur-containing proteins. An important advance in technique occurred when Brdicka (1938; 1939) demonstrated that filtrates prepared from alkali-treated sera after precipitation with sulphosalicylic acid showed much greater differences between normal sera and sera from cancer patients. With the filtrates the difference between the two types of sera was reversed, that is, the sera from cancer patients showed higher waves, indicating the presence of more sulphidic substance in their filtrates.

Further work showed that the presence of a high filtrate wave was not specifically characteristic of cancer sera (Brdicka, 1947). A valuable observation was, however, that in sera from patients undergoing successful treatment (e.g. surgical or irradiation therapy) the abnormally high serum filtrate waves reverted to normal size.

Recent increasing use of chemotherapeutic measures, at least in some forms of malignancy, suggested that the polarographic method might have some value as an objective, albeit purely empirical, measure of the efficacy of such therapy.

With this object in view some preliminary investigations were carried out which have already been briefly reported (Robinson and Warren, 1948). A larger series of sera from normal subjects, from patients with malignant and non-malignant diseases and sera from patients with malignant disease undergoing therapy, has now been studied in an attempt to assess the value of the method and its limitations. The results reported in the present paper are based on the polarographic examination of approximately 300 sera.

EXPERIMENTAL.

The technique was standardized for use with a Tinsley ink-recording polarograph. The cell was of a simple cylindrical type, open to air. The anode consisted of a pool of mercury of 1–2 cm. diameter. The capillary had the following characteristics: At a pressure of mercury of 71·5 cm. the drop-time was 3·57 seconds (open circuit), and the weight of mercury dropping per second was 1·63 mg. All polarograms were measured at room temperature (15–20° C.) since preliminary observations indicated that the temperature coefficient of the diffusion current was very small.

Blood was collected by venepuncture and allowed to clot. Serum was removed and treated as follows: Serum (0·4 ml.) was diluted with N/10 potassium hydroxide solution (1·0 ml.) and allowed to stand at room temperature for 45 minutes. Proteins were then precipitated by the addition of 20 per cent sulphosalicylic acid solution (1·0 ml.). The tubes containing the precipitated serum were well shaken, and allowed to stand for exactly 10 minutes before filtering through Whatman filter-paper No. 50. The filtrate (0·5 ml.) was added to 5·0 ml. of a buffer of the following composition: N ammonia, N/10 ammonium chloride, M/1000 cobaltihexammine chloride. In order to render the solution oxygen-free, 3 ml. of a freshly-prepared saturated solution of sodium sulphite was incorporated in each 100 ml. of buffer.

Polarograms were run between 0·8 and 1·8 volts with the instrument set at a sensitivity of 50 microamperes. The characteristic waves appeared as rounded maxima having their greatest height at a cathodic potential of about — 1·45 volts. (These waves are different in shape from those shown in publications by Brdicka and his collaborators, since the use of N ammonia instead of the N/10 originally employed by the Czech workers develops rounded maxima instead of the double waves illustrated by them.)

The general nature of the records obtained is illustrated in Fig. 1. Fig. 1 (*b*) shows a current-voltage curve for the cobalt-containing buffer solution alone. The pronounced maximum developed at about — 1·2 volts is produced by the reduction of cobalt ions. The final steep rise in the region of — 1·7 volts corresponds to the discharge of ammonium ions. Fig. 1 (*c*) shows the current-voltage curve for the same buffer solution to which serum filtrate has been added. The maximum on the cobalt curve has been almost entirely suppressed, and the wave for the cobalt ions is now succeeded by a new wave with a peak at approximately — 1·45 volts. The second wave is produced by the interaction of — SH containing molecules and cathodic electrons. The height of this filtrate wave (in microamperes) is taken as the peak height measured from a base line drawn through the point of inflexion between the end of the cobalt wave and the beginning of the filtrate wave. As will be seen in later figures, the actual shape of the current-voltage curve at this point of inflexion is variable but, in practice, there is no difficulty in determining its position with a fair degree of accuracy. The filtrate waves encountered in the present experiments varied in height from a few microamperes (with normal sera) to about 40 microamperes (with pathological sera). With the sensitivity of the instrument set at 50 microamperes (i.e. with the full range of the current axis of the recording chart equivalent to 50 microamperes) all recordings could be made on the same scale, and direct comparisons of sera obtained on different occasions from the same patient facilitated.

RESULTS.

Normal sera.

Sera from 20 normal males gave waves varying in height from about 4 to 10 microamperes, with a mean value of 6·5 microamperes. Repeat observations on the same individual at different times showed little variation. In a series of normal women there was a range of 2 to 8 with a mean of 6·0 microamperes. The type of current-voltage curves obtained with filtrates from normal sera is illustrated in Fig. 2, which shows 8 such curves for normal male sera.

Pathological sera.

Most of the sera examined were from patients suspected of cancer. A smaller number of cases on confirmed non-malignant conditions were also investigated for comparison. In the interpretation of polarograms from pathological sera a value of 10 microamperes was taken as representing the upper limit of normality so far encountered in this work. This arbitrary limit was taken for want of a

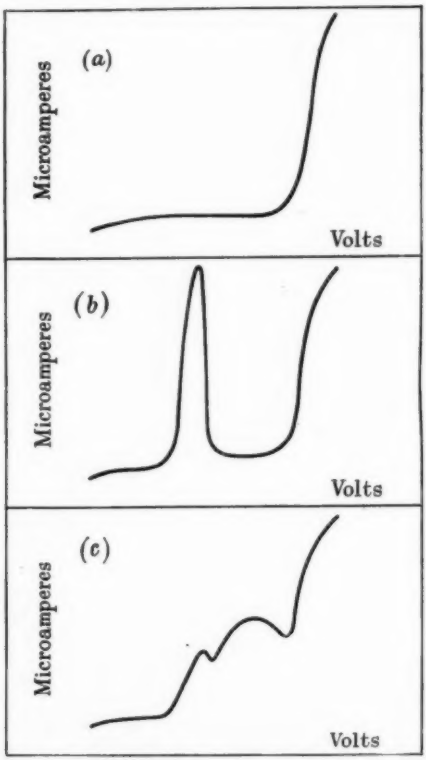

FIG. 1.—Polarographic tracings.
(*a*) Ammonia-ammonium chloride buffer.
(*b*) Buffer plus cobaltihexammine chloride.
(*c*) Cobaltic buffer plus blood filtrate.

better but, since the wave height for a normal serum can be as low as 2 microamperes in some individuals, values less than 10 microamperes might represent deviations from normality in some cases. However, for the present purpose, all values under 10 are considered as within normal limits.

Stomach and duodenum.—Twenty-five cases were examined in which the provisional diagnosis lay between neoplasia or gastric or duodenal ulcer. Seven confirmed cases of carcinoma of the stomach gave waves ranging from 12 to

24 microamperes. Confirmed gastric ulcers (11 cases) gave values from 5 to 22 microamperes, and 6 of these cases lay above the limit of 10 microamperes. Seven cases were confirmed as duodenal ulcers; 1 serum showed a wave less than 10 microamperes, while 6 ranged up to 21 microamperes.

Bronchus.—In 10 suspected cases of carcinoma of the bronchus 8 sera gave wave heights exceeding 10 microamperes. In all but one of these cases the diagnosis was confirmed. The 2 sera giving values below 10 were subsequently shown to be free from malignancy.

Large intestine and rectum.—Of 11 cases examined, 5 were later found to have carcinoma of the colon, 5 carcinoma of the rectum and 1 carcinoma of the caecum. Sera from 10 of these cases gave waves greater than 10. One case of rectal carcinoma was 7·5.

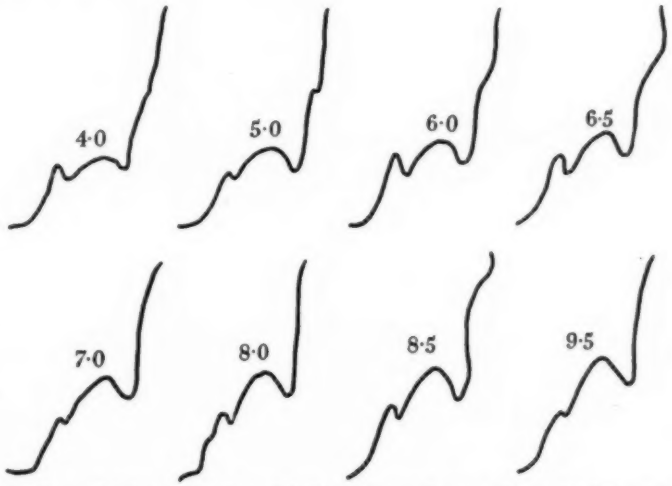

FIG. 2.—Tracings of polarograms from filtrates of normal sera. Wave heights are marked in microamperes.

Prostate.—Thirty-four cases of prostatic disease were examined. Sera from 7 patients with benign hypertrophy of the gland gave waves varying from 5 to 10 microamperes, that is, all fell within the normal range. All sera from cases of carcinoma showed values in excess of normal, the lowest being 11·5 and the highest 30. One serum from a patient with prostatic abscess had a wave height of 24.

The values just mentioned were obtained on patients not receiving any form of therapy. Most of the carcinoma cases were subsequently treated with synthetic oestrogens—stilboestrol in the majority of cases. Sera from such treated cases were examined at intervals throughout the course of therapy. In general, administration of the oestrogen resulted in a decrease of wave height. The rate and magnitude of this decrease varied in different cases. Usually, under continuous therapy, the wave reverted to normal height in a few weeks. Continued increase in wave height during therapy was taken as an indication for

increased dosage of oestrogen. Individual data for these cases, together with some values for serum acid phosphatase determined on the same specimens, are shown in Table I. Tracings of polarograms obtained during therapy of Case 8 are shown in Fig. 3.

TABLE I.—*Wave Heights and Acid Phosphatase Values of Sera from Cases of Prostatic Carcinoma.*

Case No.	Diagnosis.	Oestrogen therapy.	Wave height.	Acid phosphatase.
1	Benign	None	10	..
2	,,	,,	9	..
3	,,	,,	5	..
4	,,	,,	8	..
5	,,	,,	10	..
6	,,	,,	9	..
7	,,	,,	6	..
8	Carcinoma	,,	15	>100
		,,	24·5	>100
		2 weeks	9·5	..
		5 ,,	6	..
		9½ ,,	5	17
		15½ ,,	7·5	9·5
		17½ ,,	5	6·3
		21½ ,,	5	..
		34 ,,	9·5	..
9	,,	None	24	1·8
		3 weeks	14	..
		4 ,,	13	..
		5 ,,	16	..
		6 ,,	14	..
		12 ,,	9	1·3
10	,,	None	22	..
		4 days	24	21
		9 ,,	15	..
		12 ,,	13	..
		6 weeks	9	1·2
11	,,	4 months	8	3
		5 ,,	7	1·5
		6 ,,	8	1·6
		10 ,,	6	1·1
12	,,	None	30	6·4
		5 days	18	..
		9 ,,	14	..
		5 weeks	9	1·2
13	,,	5 months	20·5	7·5
		6 ,,	16	1·6
		7 ,,	10	2·5
		10 ,,	15	3·6

TABLE I—(*cont.*).

Case No.	Diagnosis.	Oestrogen therapy.	Wave height.	Acid phosphatase.
14	Carcinoma	None	12	..
		,,	17	..
		1 week	10	..
15	,,	None	25·5	..
		6 weeks	12	..
16	,,	None	11·5	..
17	,,	,,	19	..
18	,,	,,	16·5	..
19	,,	,,	12	..
20	,,	,,	16	..
21	,,	,,	17·5	..
22	,,	,,	11·5	..
23	,,	,,	24	..
24	,,	,,	15	..
25	,,	+	7	..
26	,,	+	10	..
27	,,	+	10	..
28	,,	5 days	25	1·5
29	,,	7 weeks	8	..
30	,,	7 months	12	..
31	,,	19 ,,	3	..
32	,,	1 week	4	..
33	,,	11 months	5	..
34	Abscess	..	24	..

(A plus sign in the oestrogen therapy column indicates that the patient was undergoing therapy but that the date of commencement was uncertain.)

Miscellaneous carcinomata.—In this group are included sera from patients with carcinoma of bladder, gall bladder, kidney, pancreas, parotid, thyroid, tongue, oesophagus and ovary. In all these cases, with the exception of the serum from the patient with the parotid tumour, waves exceeding 10 microamperes were recorded.

Generalized malignant conditions.—Hodgkin's disease showed extremely high waves. Two cases gave values of 32 and 39 microamperes. Of 4 cases of myelomatosis, 2 exceeded 10 and 2 fell within the normal range. A case of reticulosarcoma showed a wave of 12, one of lymphadenoma 27 and one of lymphatic leukaemia also 27. Two cases of generalized carcinomatosis had wave heights of 17 and 27.

Non-malignant conditions.—In all cases investigated which were free from infection or inflammation waves were recorded which fell within normal limits. In pulmonary tuberculosis, on the other hand, high waves were obtained—sometimes extremely high, e.g. 35 microamperes.

In Fig. 4 the wave heights recorded with sera from some typical conditions are illustrated diagrammatically.

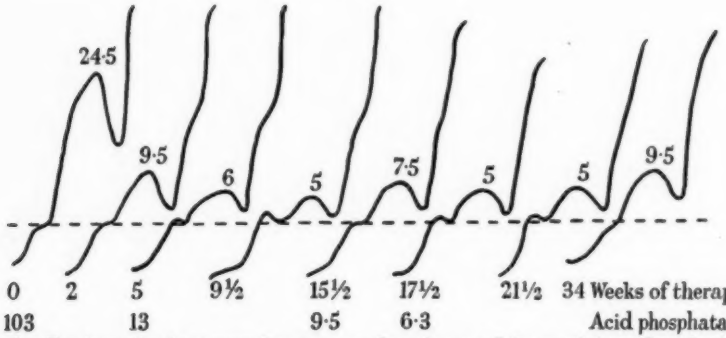

FIG. 3.—Tracings of polarograms from a case of carcinoma of the prostate undergoing treatment with stilboestrol. Wave heights in microamperes. Acid phosphatase in King-Armstrong units per 100 ml. blood.

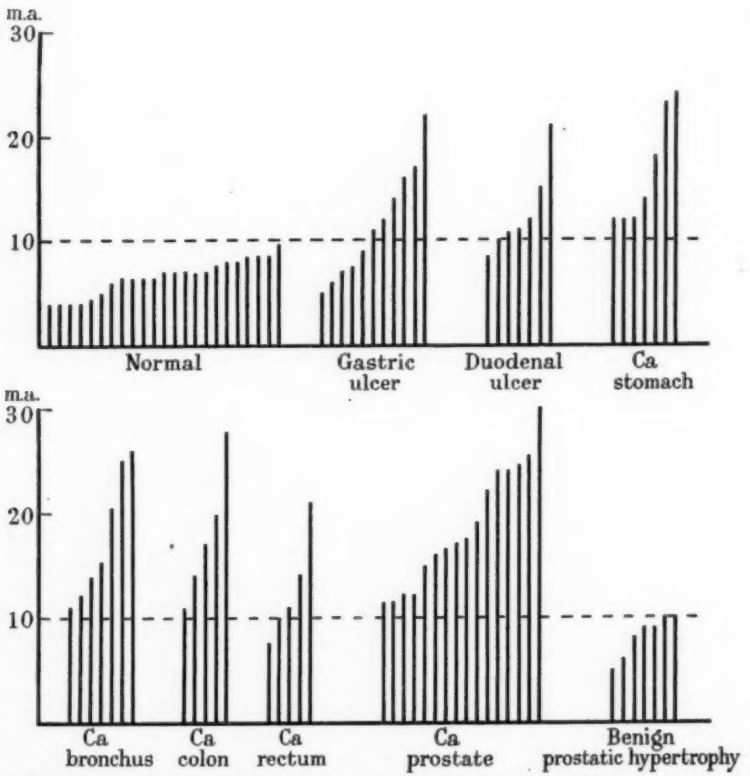

FIG. 4.—Wave heights in microamperes of polarograms in some typical benign and malignant conditions.

DISCUSSION.

As Brdicka has repeatedly emphasized, a single polarographic examination of a serum cannot give definite proof of malignancy, since abnormally high waves are frequently encountered in non-malignant diseases. However, if infection or inflammation can be excluded, wave heights in excess of normal indicate that malignancy may be present, and suggest the advisability of exhaustive search for the neoplasm. The results obtained in the present work bear out this contention. In one group of patients that was investigated, the presenting symptoms were anaemia of unknown origin and loss of weight. Three such cases gave waves of 21·5, 17 and 14 on first examination. Further investigation revealed the presence of a carcinoma of the ovary in the first and of carcinoma of the colon in the second and third respectively.

As an aid to diagnosis the most useful method is undoubtedly serial examination of sera. Repeated examination at intervals of a week to a month may give valuable information, and permit differentiation between a benign and a malignant condition. For example, one case of gastric ulcer was given routine medical treatment. At the beginning of treatment the wave height was 22, after 24 days of treatment it was 13, and after 38 days, 8·5, that is, within normal limits. Sera from unoperated cases of carcinoma of the stomach show an increase of wave height with time.

The polarographic method appears to be particularly suitable for investigation of prostatic disease. In cases so far examined the presence of malignancy has invariably led to high waves, while benign enlargement of the gland has not. Moreover, the height of the wave is decreased by the administration of oestrogens. A larger series than the one reported in the present paper is clearly necessary before definite conclusions can be drawn, but if further investigation shows that the wave height is correlated with the clinical condition of the patient, then such measurements may prove to be the most sensitive indications available of the efficacy of oestrogen therapy in individual cases.

Figures for serum acid phosphatase determinations in some cases are included in Table I. From the data for Case 8 it can be seen that the general trend of wave heights and acid phosphatase values during therapy is similar. However, low values of serum acid phosphatase do not preclude high waves (e.g. Cases 9, 12, 13 and 28). Correlation between the polarographic and enzyme measurements is not absolute, and further work is required to determine which method gives a more accurate reflection of the clinical condition of the patient. In particular, information is required as to whether cases in which the decline in wave height with continued therapy is slight, or slow (e.g. Case 13), are characterized by resistance to oestrogen, and have a worse prognosis than those (e.g. Case 10) in which the decline is marked or rapid.

Very high waves are encountered in Hodgkin's disease. One such case was examined during therapy with nitrogen mustard. The initial wave of 32 declined to 26 after one week of treatment, but had reverted to the original value of 32 when death occurred 6 weeks later.

A number of cases of breast carcinoma and mastitis was also examined, but the series is not yet large enough for detailed report. The preliminary results were in agreement with those reported by Fort, Brdicka, Ott and Voriskova

(1942), namely, that the benign conditions were usually characterized by normal waves, but that a proportion of malignant cases also had waves within normal limits. In general, however, the higher grades of malignancy were associated with higher waves.

The preliminary survey recorded in the present paper indicates that the examination of sera by polarographic means in suitably selected cases may be a useful aid to diagnosis, and that the use of this method in the assessment of the results of therapy merits further study.

SUMMARY.

1. Brdicka's method of polarographic examination of pathological sera is described.

2. Results of the application of this method to the examination of a number of malignant and non-malignant conditions are recorded.

3. The method is particularly suited to the examination of sera from patients with prostatic disease. Repeated examination of such sera gives a valuable indication of the effectiveness of therapy with synthetic oestrogens in cases of carcinoma of the prostate.

4. It is suggested that the method may have wider uses as an objective measure of the results of chemotherapy in other forms of cancer.

This work has been supported by generous grants to this Hospital by the British Empire Cancer Campaign. I am grateful for much helpful co-operation and advice from the Medical Staff of St. Bartholomew's Hospital. I am particularly indebted to Dr. W. G. Dangerfield for obtaining many of the blood specimens, to Mr. R. Hudson for determinations of acid phosphatase, and to Dr. F. L. Warren for the use of his polarograph in the initial stages of this work.

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THE EFFECT OF ACRIDINE DERIVATIVES ON GROWTH AND MITOSIS OF CELLS *IN VITRO*.

I. LASNITZKI* AND J. H. WILKINSON.

From the Strangeways Research Laboratory, Cambridge, and the Department of Chemical Pathology, Westminster Medical School (University of London), 17, Horseferry Road, London, S.W.1.

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In view of the recent developments in the chemotherapy of acridines it seemed desirable to extend the study of Dustin (1925) and Bucher (1939) on the antimitotic action of 2:8-diamino-10-methylacridinium chloride (trypaflavine) to a number of new acridine derivatives. The effect of the following compounds on cells growing *in vitro* has therefore been examined :

5-aminoacridine hydrochloride,
5-amino-10-methylacridinium bromide,
5-amino-1-phenylacridine hydrochloride,
5-amino-1-phenyl-10-methylacridinium bromide, and
2-chloro-5-amino-10-methylacridinium bromide.

Tissue cultures which are simple living units without a blood or nerve supply are ideal material on which to demonstrate the direct effect of chemical or physical agents upon the cell. Owing to the simplification and standardization of experimental conditions which the method provides it is also possible to obtain precise quantitative data of the effect.

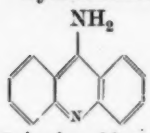
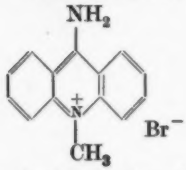
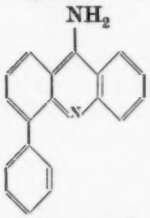
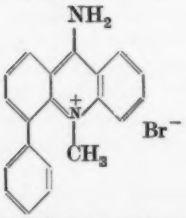
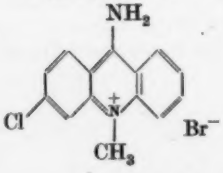
TECHNIQUE.

The cultures used for this work were obtained from the sclerotic of 11-day-old chick embryos, and grown by the hanging-drop technique in a medium consisting of equal parts of chick plasma and chick embryo extract. Two subcultivations were made before carrying out the experiment. Various concentrations of the acridine derivatives were then added to the medium of the experimental cultures, while the control cultures received an equal amount of Tyrode solution. The final concentrations of each compound in the culture media are listed in Tables I and II. For each concentration of the compounds six to eight experimental and six to eight control cultures were used.

After 24 hours' incubation, i.e. at the time when the number of cell divisions normally reaches a maximum, the experimental cultures showing growth and all the controls were fixed in Susa solution and stained with haematoxylin. All mitotic cells present in the zone of growth were counted in the treated and untreated cultures, and the results were expressed quantitatively as the percentage inhibition of mitosis in the treated cultures as compared with the controls (Table II). In cultures treated with 5-amino-1-phenyl-10-methylacridinium

* Sir Halley Stewart Research Fellow.

TABLE I.—*The Effect on Outgrowth of Aminoacridines in Treated Cultures.*

	Concentration.											
	1:30,000.		1:60,000.		1:120,000.		1:240,000.		1:480,000.		1:960,000.	
	Number of cultures in which outgrowth is—											
	Absent.	Present.	Absent.	Present.	Absent.	Present.	Absent.	Present.	Absent.	Present.	Absent.	Present.
5-Aminoacridine hydrochloride	6	0	6	0	6	0	7	0	5	1	4	3
 , HCl												
5-Amino-10-methylacridinium bromide	6	0	6	0	5	1	4	2	..	..	..	..
												
5-Amino-1-phenylacridine hydrochloride	6	0	6	1	2	5	..	..	..	..	..	..
 , HCl												
5-Amino-1-phenyl-10-methylacridinium bromide	2	6	0	6	0	6	..	..	..	..	..	..
												
2-Chloro-5-amino-10-methylacridinium bromide	6	0	6	0	6	0	5	1	0	6	..	..
												

bromide at 1/30,000 and 1/60,000 the incidence of cell degeneration was determined by counting resting and degenerating cells in several comparable fields of experimental and control cultures. The result was expressed as a percentage of resting cells. The values shown in Table II represent the increase of degenerate cells in treated cultures as compared with the controls.

TABLE II.—*Showing Percentage Inhibition of Mitosis in Treated Cultures as Compared with Control Cultures.*

	Concentration.					
	1/30,000.	1/60,000.	1/120,000.	1/240,000.	1/480,000.	1/960,000.
5-Aminoacridine hydrochloride	100	100	100	100	58 ± 8·5	51 ± 8·4
5-Amino-10-methylacridinium bromide	100	100	100	75 ± 4·8	..	..
5-Amino-1-phenylacridine hydrochloride	100	83 ± 1·5	61 ± 2·1	..	..	..
5-Amino-1-phenyl-10-methylacridinium bromide	93 ± 1·7	54 ± 4·5	0 ± 7·9	..	..	..
	Increase of degenerate cells 10% ± 3·2	Increase of degenerate cells 21% ± 4·5				
2-chloro-5-amino-10-methylacridinium bromide	100	100	100	100	3 ± 6·2	..

RESULTS.

The growth of tissue cultures is due to two factors: cell migration and mitosis. In the type of culture used cells begin to wander out of the explant after a few hours' incubation: mitosis begins several hours later and reaches a maximum about the 24th hour, by which time a zone of new tissue consisting of resting, dividing cells and, as a rule, a few degenerate cells, has formed around the explant.

In the higher concentrations all five compounds caused a considerable disturbance of cell migration. In many cultures growth was entirely absent or only a few cells protruded from the explant, while in others the zone of growth was substantially reduced (Table I, Fig. 1–6).

All the five compounds tested had also a marked antimitotic action of varying degree. The greatest effect both on migration and mitosis was seen in cultures treated with 5-aminoacridine hydrochloride. There was no outgrowth at all at

concentrations greater than 1:240,000. At 1:480,000 five of the six treated cultures showed no outgrowth, and in the sixth there was a mitotic inhibition of 58 per cent. The compound was still effective at a concentration of 1:960,000; only four of the seven treated cultures had normal outgrowth, and mitosis was reduced to half the normal value.

The introduction of a quaternary methyl group into the 5-amino-acridine molecule considerably reduced the growth inhibitory effect. Thus in four of the six cultures treated with 5-amino-10-methylacridinium bromide at a concentration of 1:240,000 no outgrowth was observed, and the remaining two showed an inhibition of 75 per cent of mitosis. The presence of the chlorine atom in 2-chloro-5-amino-10-methylacridinium bromide appeared to counterbalance the effect of the methyl group, for at the same concentration (1:240,000) five of the six experimental cultures lack a zone of growth and, moreover, cell divisions were absent in the sixth which did show migration.

The presence of a 1-phenyl group caused a much more pronounced diminution of the growth inhibitory effect, for 5-amino-1-phenylacridine hydrochloride proved less active at 1:120,000 than the parent compound at 1:960,000. A summation of the effects of the 1-phenyl and 10-methyl groups seemed to occur in the case of 5-amino-1-phenyl-10-methylacridinium bromide. The outgrowth was very little affected by this substance. At 1:30,000 only two of the eight treated cultures showed absence of growth, but in the remaining six mitosis was very scarce, with 93 per cent inhibition. At 1:60,000 all cultures had a zone of growth with mitosis reaching half the normal value, while at 1:120,000 both outgrowth and mitosis were normal (Tables I and II).

The incidence of abnormal mitosis was not significantly increased in the treated cultures. This suggests that once the cells entered division they were able to complete it successfully in contrast to cells treated with colchicine (Dustin, 1939).

Hyperchromatosis and pycnosis of the nuclei similar to the cell degeneration seen and described in cells *in vitro* after radiation (Lasnitzki, 1940) were present

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EXPLANATION OF PLATES.

FIG. 1–6 show growing edge of cultures fixed at 24 hours' incubation. × 66.

FIG. 1.—Untreated control culture.

FIG. 2.—Culture treated with 5-amino-1-phenyl-10-methylacridinium bromide at 1/30,000.

FIG. 3.—Culture treated with 5-amino-1-phenylacridine hydrochloride at 1/120,000.

FIG. 4.—Culture treated with 5-amino-10-methylacridinium bromide at 1/240,000.

FIG. 5.—Culture treated with 2-chloro-5-amino-10-methylacridinium bromide at 1/240,000.

FIG. 6.—Culture treated with 5-aminoacridine hydrochloride at 1/480,000.

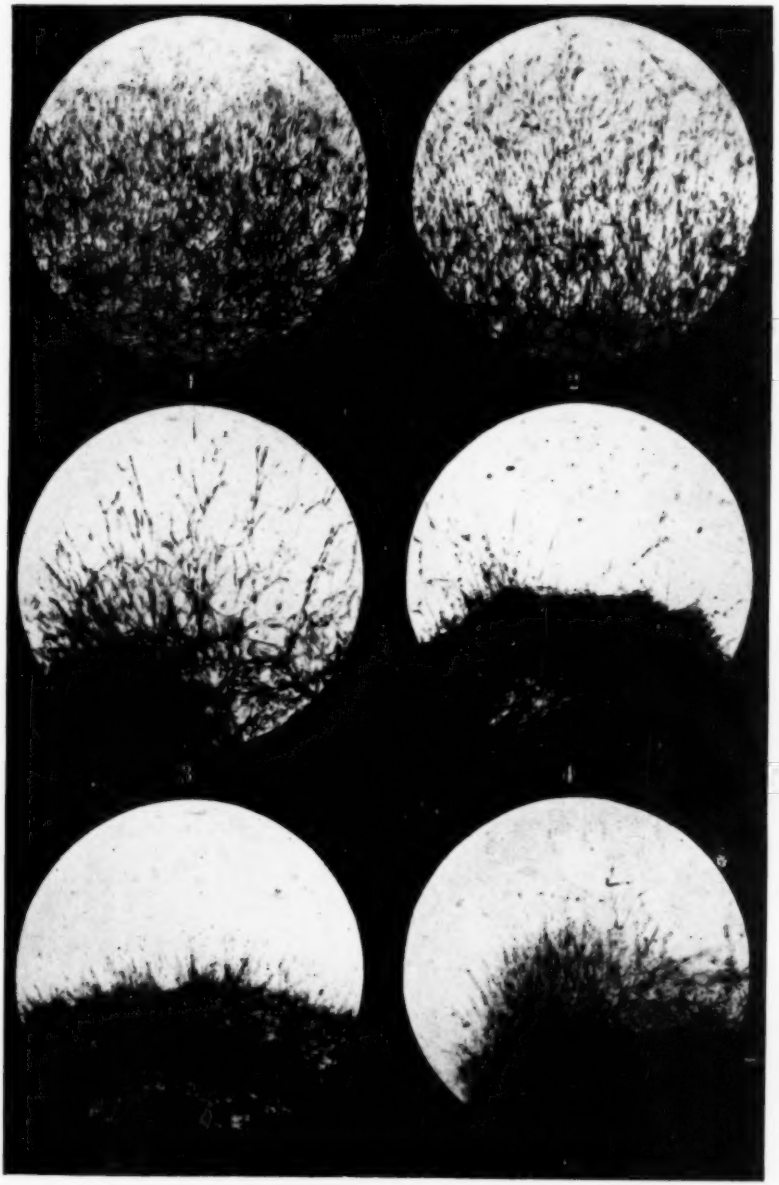
FIG. 7–10 show groups of fibroblasts in normal and treated cultures fixed at 24 hours' incubation. × 1000.

FIG. 7.—Normal fibroblasts.

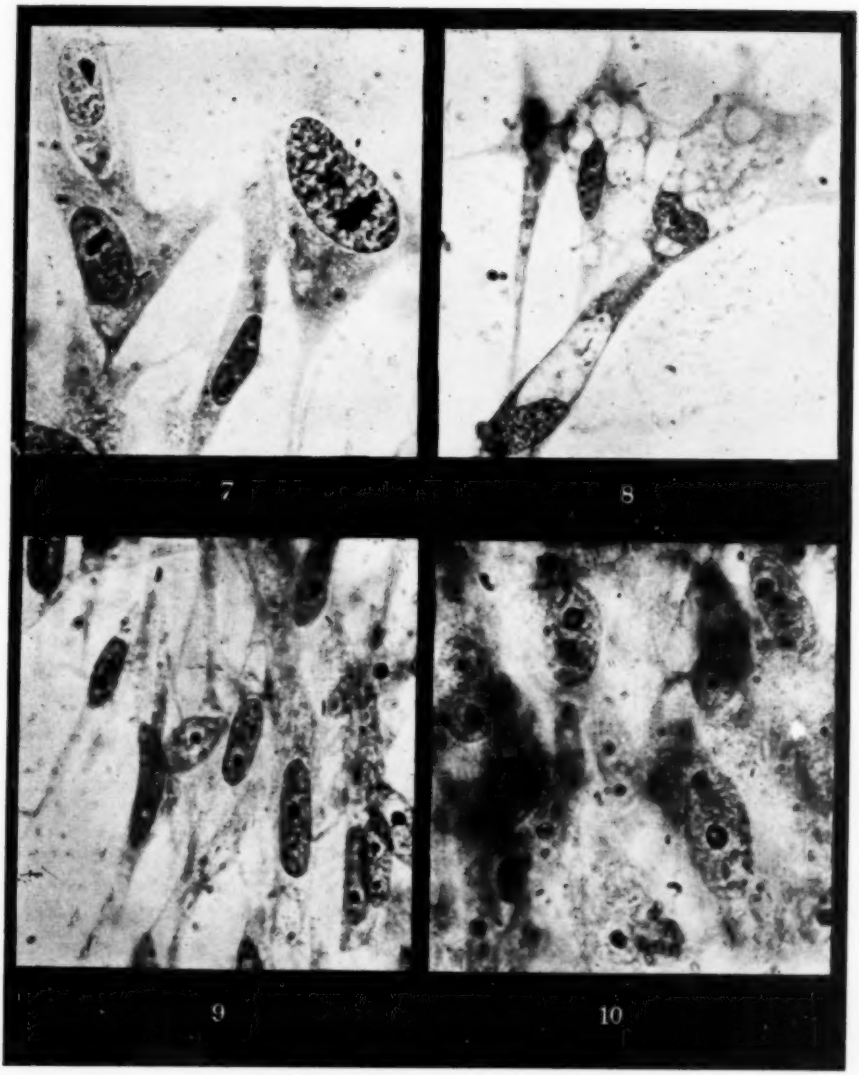
FIG. 8.—Abnormal vacuolization in fibroblasts treated with 5-amino-1-phenyl-10-methylacridinium bromide at 1/30,000.

FIG. 9.—Shrinkage of nucleoli in cells treated with 2-chloro-5-amino-10-methylacridinium bromide at 1/240,000.

FIG. 10.—Vacuolization of nucleoli in cells treated with 5-aminoacridine hydrochloride at 1/480,000.



Lasnitzki and Wilkinson.



7 8

9 10

Lasnitzki and Wilkinson.

in the experimental cultures. Degenerate cell counts in cultures treated with 5-amino-1-phenyl-10-methylacridinium bromide at 1/30,000 and 1/60,000 showed a marked increase over that of the controls at both concentrations (Table II).

The resting cells of the treated cultures were narrower and more pointed than the normal fibroblasts of the controls, and they did not stain as deeply with haematoxylin. Abnormal vacuolation was frequently observed in the cytoplasm Slightly affected cells showed one distinct vacuole in the region of the centrosphere, while in others a number of vacuoles could be seen. These finally coalesced to form one large vacuole occupying the whole cytoplasm (Fig. 8). In many cells the nucleoli appeared severely damaged: they were shrunk, rounded and stained excessively with haematoxylin. Around the shrunken nucleoli vacuoles appeared. In other cells the nucleoli formed ring-like structures with a central vacuole (Fig. 9, 10).

DISCUSSION.

The diminution of activity caused by the introduction of a 1-phenyl group into the 5-aminoacridine molecule recalls the work of Albert, Rubbo, Goldacre, Davey and Stone (1945), who observed that in the acridine series bacteriostatic activity is paralleled by the degree of ionization of the molecule at the pH of the medium. The presence of the electron-attracting phenyl group in close proximity to the ring nitrogen atom weakens the basic strength of the compound, a fact determined experimentally by these workers. Its percentage ionization at the biologically important pH is therefore correspondingly reduced. In accordance with their theory 5-amino-1-phenylacridine proved to have weaker antibacterial properties than 5-aminoacridine. It appears possible that some similar rule may apply to the antimitotic properties of the series, but confirmation of this suggestion must await the accumulation of additional data.

Rubbo, Albert and Maxwell (1942) have pointed out that at low concentrations the action of the acridine antiseptics is bacteriostatic rather than bactericidal, and the present work suggests that under such conditions this is probably due to the interference with cell division and not to a general toxic effect.

The observation that the incidence of abnormal mitosis was not significantly increased in the treated cultures indicates that the effect of the compounds tested is to *prevent* cells from *entering* mitosis rather than to interfere with the actual process of division.

The frequency and degree of the nucleolar changes go roughly parallel with the antimitotic activity of the compounds examined, and it is possible that the inhibition of cell division may be due to the disturbance of the nucleoli. On the other hand, the nucleolar damage may only accompany the other changes.

The increase in degenerate cells is considerably more marked in cultures treated with 5-amino-1-phenyl-10-methylacridinium bromide at 1:60,000, and showing less mitotic inhibition than in cultures which had received twice the amount of the compound. This finding suggests that the action of the acridines at the higher dilutions is not due to a direct toxic effect on the cells, but that the appearance of degenerate cells is linked with mitotic inhibition and due to a breakdown of premitotic cells attempting division, a sequence of events observed in cultures exposed to radiation (Lasnitzki, 1943).

EXPERIMENTAL.

A commercial specimen of 5-aminoacridine hydrochloride was used in this work, and the samples of 5-amino-10-methylacridinium bromide (Albert and Ritchie, 1943) and its 2-chloro derivative (Wilkinson and Finar, 1947) were prepared by the literature methods.

5-Amino-1-phenylacridine hydrochloride was prepared from the corresponding base (Albert and Gledhill, 1945). It crystallized from water in sparingly soluble fine lemon-yellow prisms, m.p. over 300°. (Found: N, 8·8; Cl, 10·95. $C_{19}H_{14}N_2$, HCl, H_2O requires N, 8·65; Cl, 10·95 per cent.)

5-Amino-1-phenyl-10-methylacridinium bromide.—5-Amino-1-phenyl-acridine (1 g.) was heated at 105° for 30 minutes with acetic anhydride (4 c.c.) and the cooled mixture was poured into benzene (20 c.c.). The precipitated 5-acetamido-1-phenylacridine (0·97 g.) was collected by filtration and crystallized from alcohol or pyridine. It separated in pale yellow needles, m.p. 284°. The acetyl derivative (0·5 g.) was dissolved in nitrobenzene (5 c.c.), and heated with dimethyl sulphate (0·7 g.) at 140° for 30 minutes. The cooled mixture was poured into benzene (30 c.c.) and set aside overnight. The gummy product was separated by decantation and hydrolysed by heating with 48 per cent hydrobromic acid (5 c.c.) on a steam bath for one hour. The required *5-amino-1-phenyl-10-methyl-acridinium bromide* which separated on cooling crystallized from water in yellow prisms, m.p. over 300°. (Found: N, 7·65. $C_{20}H_{17}N_2Br$ requires N, 7·7 per cent.) An aqueous solution gave no precipitate with N sodium bicarbonate, but sodium hydroxide gave a yellow precipitate of the corresponding *pseudo* base.

SUMMARY.

Chick fibroblasts were grown in a medium containing a number of new acridine derivatives and their effect on outgrowth and mitotic rate was examined.

The following compounds which were added to the culture medium in concentrations ranging from 1:30,000 to 1:960,000 were used:

5-aminoacridine hydrochloride,
5-amino-10-methylacridinium bromide,
5-amino-1-phenylacridine hydrochloride,
5-amino-1-phenyl-10-methylacridinium bromide,
2-chloro-5-amino-10-methylacridinium bromide.

All the substances caused disturbance of outgrowth and/or reduction of mitosis of varying degree. 5-aminoacridine hydrochloride proved to have the greatest and 5-amino-1-phenyl-10-methyl-acridinium bromide the smallest inhibitory effect.

At the higher concentrations rounding and vacuolization of the nucleoli and vacuolization of the cytoplasm could be observed. The effect of the compounds on mitosis was found to resemble that of small doses of radiation: cells were prevented from entering division, but there was no interference with the actual process of division in contrast to colchicine.

The bearing of the degree of ionization of the substances used on their growth inhibitory action is discussed.

The authors wish to thank Mr. G. Lenney for the photomicrographs.

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SHOPE PAPILLOMA VIRUS: REVERSION OF ADAPTATION TO DOMESTIC RABBIT BY PASSAGE THROUGH COTTONTAIL.

F. R. SELBIE AND R. H. M. ROBINSON,
From the Bland-Sutton Institute of Pathology, Middlesex Hospital, London, W.1,

AND

R. E. SHOPE,
From the Rockefeller Institute for Medical Research, Princeton, New Jersey, U.S.A.

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IN his original experiments on infectious papillomatosis of the rabbit Shope (1933) found that the disease could be transmitted in series through the cottontail rabbit, the original host, but, although virus of cottontail origin was infective to the domestic rabbit, the infection could not be transmitted in series through the domestic rabbit. In later experiments Shope (1935) recovered active virus from a number of papillomas in domestic rabbits and established several transmissible strains, of which one was subsequently reported to have reached the 14th serial passage in the domestic rabbit (Shope, 1937). Other workers have found that extracts of papillomas from domestic rabbits are only occasionally infective, and there have been no further reports of transmission beyond the second passage except that of Selbie and Robinson (1947) who have transmitted a strain of Shope papilloma virus to the 12th serial passage in the domestic rabbit. The papillomas produced by this transmissible strain, like those described by Shope (1935), behave in a manner similar to those produced by the inoculation of extracts of cottontail papillomas even to the regular development of carcinomatous changes (Selbie and Robinson, 1948), as has also previously been observed by Kidd and Rous (1940) in one of Shope's transmissible strains. The infectivity, however, of the papillomas of the transmissible strain is much lower than that

of cottontail papillomas, and so gives low and irregular yields of papillomas on passage. This factor seriously interferes with critical experiments on host-parasite relationships in this infection, especially when the carcinomatous stage has been reached. With a view to overcoming this difficulty we have inoculated the cottontail with papillomas of the 12th domestic rabbit passage in the hope that the infectivity of the transmissible strain might be raised. As will be shown here this expectation has not been realized, in that the transmissible strain has lost its adaptation to the domestic rabbit by passage through the cottontail. The results of the experiments, however, are not without interest, because they throw some light on the relationships between the virus and the host in the two species of rabbit.

EXPERIMENTAL.

On January 27, 1948, a group of papillomas of the 12th domestic rabbit passage was excised from a rabbit 35 days after inoculation of papilloma extract in an area of skin that had been treated with croton-oil. The papillomas were placed in 50 per cent glycerol and were sent from the Bland-Sutton Institute of Pathology to the Rockefeller Institute for Medical Research, Princeton, U.S.A.

Passage 13 *at Rockefeller Institute for Medical Research, Princeton.*

On February 14, 1948, 0·75 c.c. of the supernatant of an approximately 5 per cent suspension of the glycerinated domestic rabbit papillomas received from the Bland-Sutton Institute of Pathology, London, was applied to the shaven and scarified abdominal skin of a domestic rabbit and a cottontail rabbit. The cottontail had been trapped in Missouri, and careful examination indicated it to be free of naturally acquired papillomatosis. The inoculated areas of skin of both animals were free of visible papillomas when inspected on February 27, 1948. However, on March 3, 18 days after infection, the inoculated areas of skin of both the domestic and the cottontail rabbit showed many scattered tiny papillomas. These developed well on both animals, and 10 days later measured 1–2 mm. in height and diameter on the cottontail and 2–3 mm. on the domestic rabbit. They were of approximately equal number on the two animals, though, as is characteristic for the two species, those on the cottontail appeared hard, compact and pigmented, while those on the domestic rabbit were friable and pink in colour, due apparently to greater vascularity. On March 29 the inoculated area of the domestic rabbit exhibited an abundant mass of discrete and semi-confluent fleshy papillomas of 5–7 mm. in thickness, while a similar crop of somewhat smaller (3–5 mm.) but more compact papillomas were present on the inoculated area of skin of the cottontail. No papillomas were present on the cottontail except at the area of inoculation. The cottontail and the domestic rabbit were sacrificed on this day (March 29), and papillomas from both animals

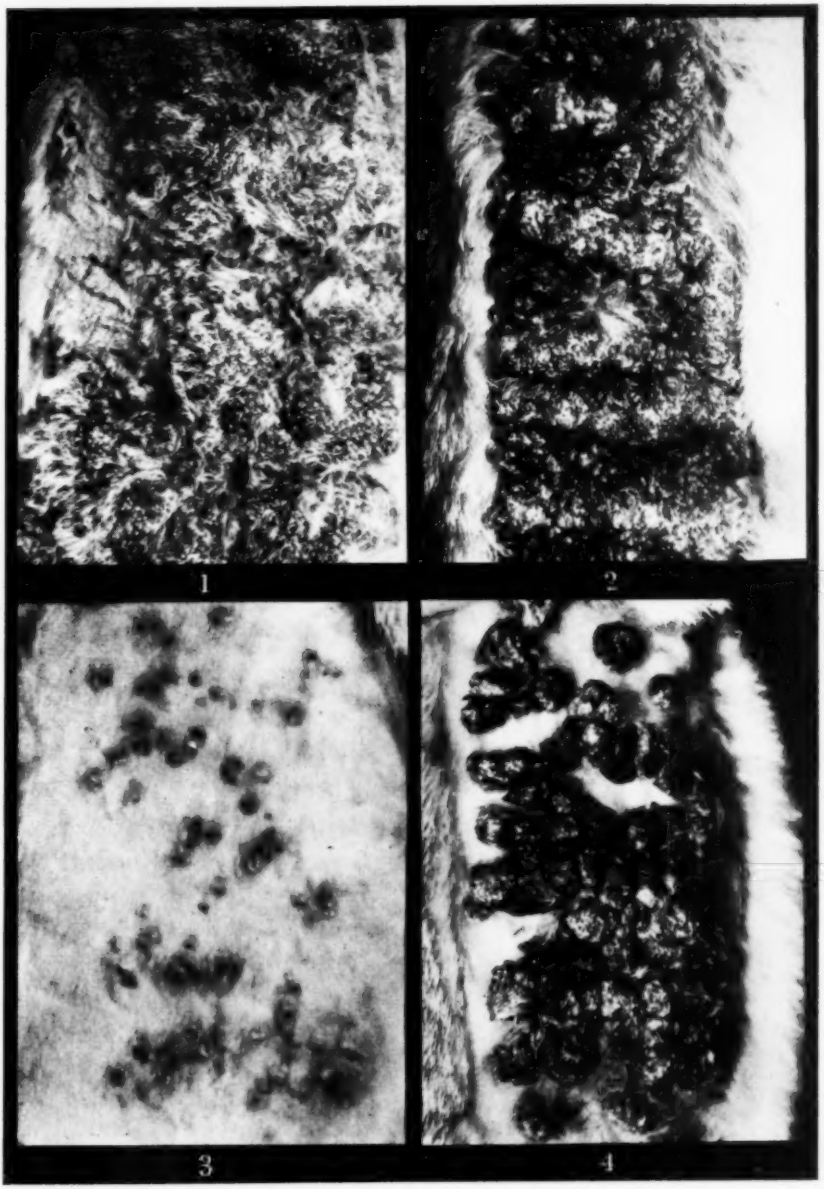
EXPLANATION OF PLATE.

FIG. 1.—Rabbit 1, Passage 14A, 25 days after inoculation with an extract of papillomas from the cottontail of Passage 13.

FIG. 2.—Rabbit 2, Passage 14A, 119 days after inoculation with an extract of papillomas from the cottontail of Passage 13.

FIG. 3.—Rabbit 3, Passage 14B, 32 days after inoculation with an extract of papillomas from the domestic rabbit of Passage 13.

FIG. 4.—Rabbit 3, Passage 14B, 119 days after inoculation with an extract of papillomas from the domestic rabbit of Passage 13.



Selbie, Robinson and Shope.

were sent in 50 per cent glycerol to the Bland-Sutton Institute of Pathology, London, where the subsequent passages were made.

On the possibility that the virus might become inactivated during its return trip from Princeton to London, two domestic rabbits were inoculated in the usual manner with each of the samples of papillomas being sent back to London. The results here coincided well with those obtained in England, indicating that the virus underwent no serious deleterious change during shipment.

The impression gained from working with the strain of papilloma virus adapted in England (Selbie and Robinson, 1947) to serial passage in domestic rabbits is that it regularly yields more abundant papillomas, and is more satisfactory as an investigational tool than the strain adapted earlier to domestic rabbits in America (Shope, 1935).

Passage 14.

On April 9, 20 per cent saline extracts of papillomas from the cottontail and domestic rabbit of the 13th passage were prepared and, using the technique described by Selbie and Robinson (1947), 10 drops of each extract were inoculated by scarification on an epilated area of skin, 12 by 8 cm., in the right flank of two domestic rabbits.

Passage 14A—*from cottontail material.*—In both rabbits that were inoculated with cottontail material hundreds of minute papillomas were seen 10 days after inoculation. The papillomas were more numerous in one rabbit (No. 1) than in the other (No. 2), and in their later development the papillomas in Rabbit 1 grew more rapidly, measuring up to 0·4 cm. at 18 days, whereas in Rabbit 2 the largest papillomas were only 0·2 cm. at this stage. On May 5, 25 days after inoculation, groups of papillomas were excised from both rabbits for passage. As is shown in Fig. 1 of Rabbit 1 at 25 days and in Fig. 2 of Rabbit 2 at 119 days, the infection was similar, both in the yield and in the appearance of the papillomas, to that produced by extracts of papillomas from the natural infection in the cottontail.

Passage 14B—*from domestic rabbit material.*—Papillomas were first observed in the two rabbits that were inoculated with domestic rabbit material on May 4, 25 days after inoculation. In one rabbit (No. 3) there were about 80 papillomas, measuring up to 1 mm. in diameter, and in the other (No. 4) there were only 15, measuring up to 0·3 mm. A week later the yields and maximum diameters of the papillomas were 150 and 0·4 cm. in Rabbit 3, and 32 and 0·2 cm. in Rabbit 4. The yield of papillomas was thus about the usual for the transmissible strain in Rabbit 4, and above the average in Rabbit 3. In Fig. 3 and 4 of Rabbit 3 at 32 and 119 days after inoculation it will also be seen that the characters and distribution of the papillomas are similar to those previously observed in earlier passages in the domestic rabbit (Selbie and Robinson, 1947). On May 20, 41 days after inoculation, a group of papi lomas was excised from Rabbit 3 for passage.

Passage 15.

Passage 15A.—On May 5, 8 domestic rabbits were inoculated with a 10 per cent extract in saline of papillomas from Rabbits 1 and 2 of Passage 14A. Four

rabbits were inoculated on four areas of skin by the methods used by Selbie and Robinson (1947) for increasing the yield and infectivity of transmissible papillomas. Two areas of skin were treated before inoculation with croton-oil; one untreated and one treated area were inoculated with papilloma extract alone, and the two remaining areas were inoculated with a mixture of papilloma extract and a suspension of another experimental infection. The infective suspension used for admixture was prepared from sheep dermatitis lesions in two rabbits, and from strawberry foot-rot lesions in the other two. In four other rabbits the papilloma extract was inoculated in four areas of skin, of which one was untreated, the second pretreated with croton-oil, the third treated with croton-oil after inoculation, and the fourth treated with croton-oil both before and after inoculation.

No papillomas were seen in any of the 8 rabbits over a period of observation of 10 weeks.

Passage 15B.—On May 15 one rabbit was inoculated in the right flank with a 10 per cent saline extract of the papillomas excised from Rabbit 3, Passage 14B. Three papillomas were observed at 23 days, and a total yield of 8 papillomas was reached by the 40th day.

DISCUSSION.

The sequence of events in the series of passages described here is shown diagrammatically in Fig. 5. It will be seen that the transmissible strain of virus papillomatosis behaved in its usual manner as regards incubation period and yield of papillomas not only during direct passage through domestic rabbits to Passage 15B, but also in the cottontail of Passage 13. On the other hand, as regards

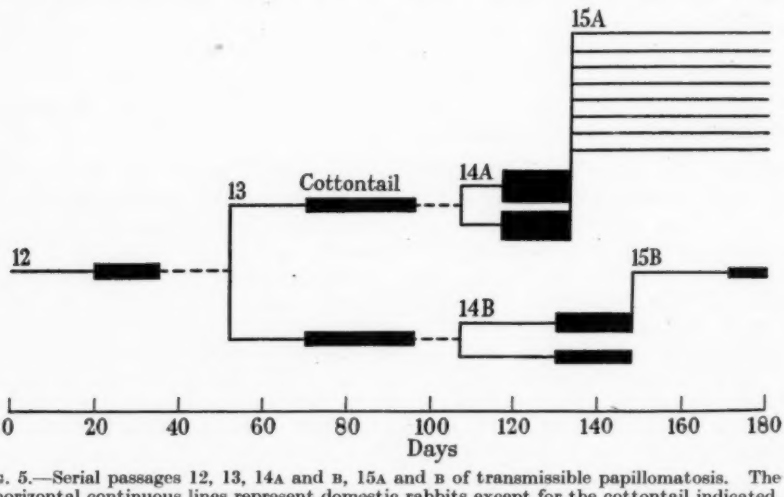


FIG. 5.—Serial passages 12, 13, 14A and B, 15A and B of transmissible papillomatosis. The horizontal continuous lines represent domestic rabbits except for the cottontail indicated. The presence of papillomas is represented by a thickening of the lines from the time papillomas first appeared to the time papillomas were removed for passage. The thickening of the lines is proportional to log $_{10}$ of the yield of papillomas. Interrupted horizontal lines indicate preservation of papillomas in 50 per cent glycerol. Vertical lines indicate source of material for passage.

infectivity, the papillomas in the cottontail of Passage 13 behaved as if they were derived from the natural cottontail infection in that they were highly infective to the domestic rabbit, giving, in Passage 14A, an infection with a short incubation period and high yield of papillomas which could not be transmitted further in Passage 15A. It is thus apparent that a single passage in the cottontail caused the transmissible strain of Shope papilloma virus to lose its adaptation to the domestic rabbit and return to its original condition.

It is evident that these results have a direct bearing on the problem presented by the apparent absence of active virus in papillomas produced in domestic rabbits by highly infective extracts of cottontail papillomas. It has been suggested by Shope (1935) that papillomas in the domestic rabbit contain a lower concentration of available virus than in the cottontail. Evidence that domestic rabbit papillomas contain much less virus has also been obtained by Beard, Bryan and Wyckoff (1939) in their demonstration of large quantities of heavy protein proportional to infectivity in papillomas from the cottontail, but not from the domestic rabbit, and by Kidd (1938*a*, 1938*b*), who, in his serological studies, has found that papillomas in the domestic rabbit contain less specific antigen and produce less circulating antibody than those from the cottontail. In view of these previous findings and the relatively low infectivity of their transmissible strain Selbie and Robinson (1947) have suggested that the fundamental difference in the nature of the infection in the two species of rabbit is that the lesion in the domestic rabbit is produced with much less multiplication of the virus in the affected cells. The inoculation of extracts of papillomas would thus usually fail to infect rabbits because of the difficulty of bringing the scanty virus particles in association with sensitive cells.

The results that have been described here and those that have already been reported are consistent with the view that there is less proliferation of virus in the domestic rabbit. On this basis the original adaptation of the virus to the domestic rabbit can be ascribed to an acquired capacity for increased multiplication in the affected cells so that the papillomas contain sufficient virus for successful passage. This adaptation, however, has not been sufficient to raise infectivity to the level found in cottontail papillomas, so that one must assume that multiplication of the transmissible virus in the papilloma cells of the adapted strain is on an intermediate level. In the passage to the cottontail the yield of papillomas, being determined by the number of infecting particles in the inoculum, has been relatively low and characteristic of the transmissible virus strain. The virus particles, however, having gained entry into the susceptible cells of the cottontail, have resumed their natural behaviour of rapid multiplication within these cells to produce highly infective papillomas as indicated by the succeeding passage. At the same time, during its sojourn in the cottontail, the virus has lost its transmissibility in the domestic rabbit, and this can be ascribed to a loss of the capacity that it gained by its original adaptation for increased multiplication in the affected cells of the domestic rabbit.

SUMMARY.

A strain of Shope papilloma virus which can be transmitted in series in the domestic rabbit has lost this property after a single passage in the cottontail rabbit.

It is suggested that the fundamental difference in the nature of the infection in the two species of rabbit is that the lesion in the domestic rabbit is produced with much less multiplication of the virus in the affected cells, and that adaptation of the virus to serial passage in the domestic rabbit can be ascribed to an acquired capacity for increased multiplication in the affected cells.

Part of the expenses of this research were defrayed by the British Empire Cancer Campaign.

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LUNG ADENOMAS INDUCED BY URETHANE IN CBA MICE.

F. R. SELBIE AND A. C. THACKRAY.

From the Bland-Sutton Institute of Pathology, Middlesex Hospital, London, W.1.

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TYZZER (1907) was the first to show that lung adenoma is a frequent spontaneous tumour in the mouse. Since then it has been found that the incidence of this tumour varies greatly in different strains of mice, approaching 100 per cent in some strains and only rarely seen in others. Several workers have shown that the incidence of lung adenomas in mice can be raised by various experimental procedures, such as repeated application of tar to numerous separate areas of skin (Murphy and Sturm, 1925), or prolonged exposure to tar-containing road dust (Campbell, 1934). The most effective method was first described by Nettleship, Henshaw and Meyer (1943), who found that weekly anaesthetic injections of urethane raised the incidence of lung adenomas in C_3H mice from 5 to over 80 per cent. In further experiments with Strong A mice, which normally have a 75 per cent incidence of these tumours at 18 months, numerous lung tumours were found in mice of $6\frac{1}{2}$ months of age that had received 14 weekly injections, whereas only one mouse showed one tumour in a similar untreated group. These authors also showed that divided subanaesthetic doses produced this effect, and it was later demonstrated that the number of tumours obtained was proportional to the number of doses of urethane, and that oral administration of urethane and subcutaneous implantation of urethane crystals was as effective as intraperitoneal injection (Henshaw and Meyer, 1944, 1945).

These workers have thus shown that urethane is an effective agent not only in greatly increasing the incidence of lung adenomas in mice that normally show few of these tumours spontaneously, but also in accelerating their appearance in strains that have normally a high incidence. In the present communication it will be shown that urethane is also effective by the intraperitoneal, oral and intranasal routes in CBA mice which normally present very few lung adenomas. Advantage has also been taken to examine the adenomas histologically in order to determine their nature and site of origin.

MATERIAL AND METHODS.

A batch of 75 male and 75 female CBA mice, 8 to 12 weeks old, was divided into 5 groups of 30, each group containing an equal number of male and female mice. Urethane was administered to 3 groups, and the 2 other groups were set aside and kept under the same conditions so that the normal incidence of tumours in the experimental period could be estimated. Urethane was used in a 5 per cent solution in distilled water, and was administered twice weekly by intraperitoneal injection in a dose of 0·2 c.c. to the first group of mice, by stomach tube in a dose of 0·4 c.c. to the second group, and by nasal instillation of about 0·05 c.c. to the third group. A considerable number of mice died from intercurrent infection, especially during the administration of urethane by stomach tube. Although records were kept of all mice dying during the experiments, they were rejected in assessing the results.

At 7 months from the beginning of the administration of urethane the surviving mice were killed. Tumours were found only in the lungs, and a count was made of the tumours that could be seen on the surface of the intact lungs. These were clearly visible as rounded opaque areas with the pleura raised up over the surface of the larger growths. Suitable pieces of the lungs were then cut and fixed in formol Zenker. From histological examination it was apparent that a number of smaller and more centrally placed tumours must have been missed in the tumour count, so that the figures shown in Table I are, if anything, an understatement.

RESULTS.

Incidence of lung tumours.

The results of the experiments are summarized in Table I, where it will be seen that lung tumours were produced in all the surviving mice that had received urethane by injection or by stomach tube, but only in 4 of 20 mice after intranasal instillation, probably because of the much smaller dosage. There was considerable variation in the number of tumours, but the average yields within groups of mice showed that oral administration was as efficient as intraperitoneal administration, and that there was no difference between the male and female mice in their sensitivity to lung tumour formation after urethane. In the untreated mice only one adenoma was found, confirming the normally low incidence of this tumour in CBA mice.

Histology.

The histological appearances agreed closely with the original description of mouse lung adenomas by Tyzzer (1907). Microscopically the tumours were seen to be circumscribed, but not encapsuled, and there was less compression of

TABLE I.—*Induction of Lung Adenomas in CBA Mice by Urethane.*

Five per cent urethane twice weekly for 5 weeks. Dose and route.		Mice surviving 7 months. Total number.	Mice surviving 7 months. No. with adenomas.	Number of adenomas per mouse. Range.	Number of adenomas per mouse. Average.	
0·2 c.c. intraperitoneal	Males	15	15	2–16	8·2	8·0
	Females	9	9	3–18	7·8	
0·4 c.c. by stomach tube	Males	3	3	4–10	7·1	10·3
	Females	6	6	1–17	11·8	
0·05 c.c. intranasal	Males	11	2	2, 8	0·9	0·8
	Females	9	2	2, 4	0·7	
None	Males	27	0	0	0	0·02
	Females	24	1	1	0·04	

the surrounding lung tissue than would have been expected from the size of the new growths; indeed, the appearances suggested that the tumour had grown by successive infiltration of fresh alveoli rather than by expansion of the main tumour mass (Fig. 1). This impression was strengthened by the study of sections stained to show the elastic fibres, in that the elastic network was continued into the tumour, though less prominent there, and was not condensed at its margin. Some of the more centrally placed tumours abutted on or surrounded bronchioles, but those at the periphery of the lung usually had no connection with bronchioles, as shown by following selected tumours through serial sections (Fig. 2). The outer part of the tumours appeared compact, but towards the centre the tumour cells were arranged on their supporting stroma to form papillary processes, so that there were numerous branching cleft-like spaces (Fig. 2).

The cubical or polygonal tumour cells had spherical or occasionally oval or indented vesicular nuclei with a well-marked chromatin network, and usually contained a single nucleolus (Fig. 3). A certain number of darker staining condensed nuclei were present, but apart from these the nuclei were uniform in size and chromatin content and mitoses were not seen. The cell outline was sharply defined, and while the cytoplasm usually stained uniformly, there were occasionally granules of black pigment within the cells, and the cytoplasm of

EXPLANATION OF PLATE.

FIG. 1.—A lobe of lung from a mouse killed 7 months after intraperitoneal administration of urethane, showing two peripheral tumours at the upper margin. Haematoxylin and eosin. × 10.

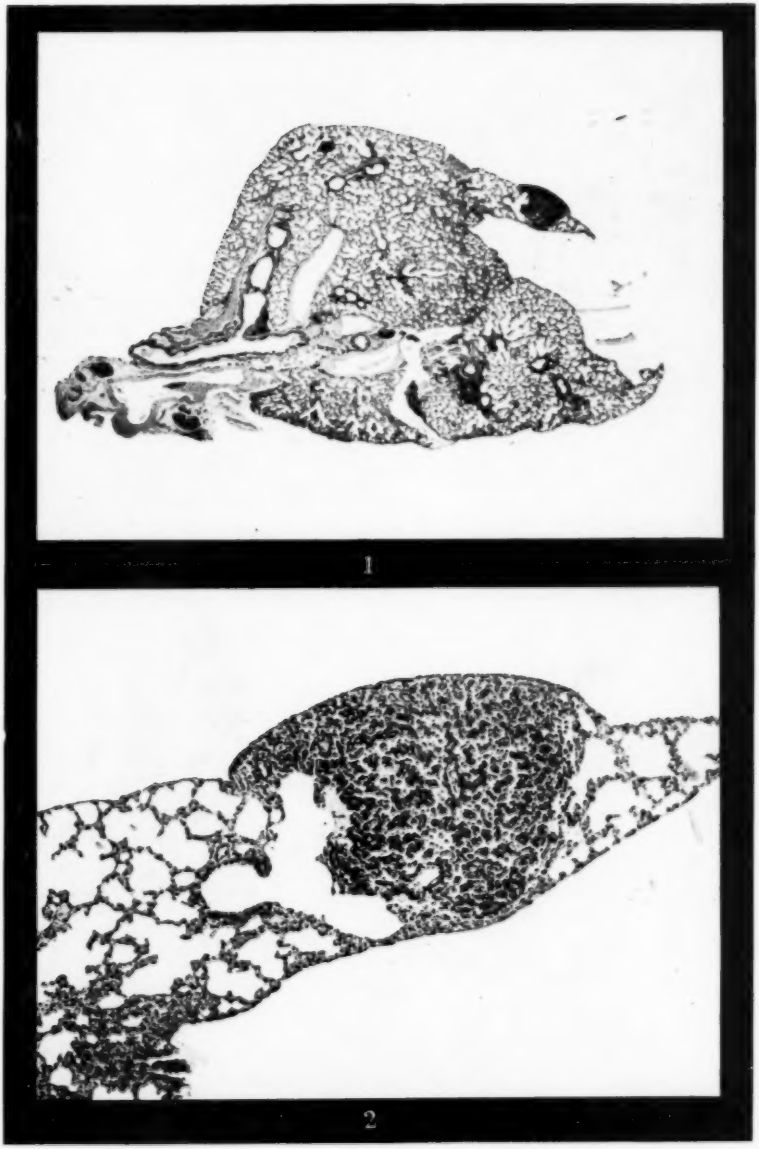
FIG. 2.—The larger tumour shown in Fig. 1. One of a series of sections which shows the nearest approach of the bronchiole to the adenoma. Haematoxylin and eosin. × 75.

FIG. 3.—A high-power view of the centre of the tumour in Fig. 2, showing the characters of the tumour cells and cleft-like spaces between the papillary processes. Haematoxylin and eosin. × 430.

FIG. 4.—Part of a tumour from a mouse 7 months after oral administration of urethane, showing foam and dust cells lying among the tumour cells. Haematoxylin and eosin. × 475.

FIG. 5.—An area at the periphery of the lung from the same mouse as in Fig. 4, showing widespread thickening of the alveolar walls, often in areas remote from the bronchioles. Haematoxylin and eosin. × 75.

FIG. 6.—A high-power view of an early stage in tumour formation from the same mouse as in Fig. 4 and 5. All stages are seen from the thin normal alveolar wall through swelling and proliferation of the epithelial cells to the first appearance of a solid group of tumour cells. Haematoxylin and eosin. × 250.



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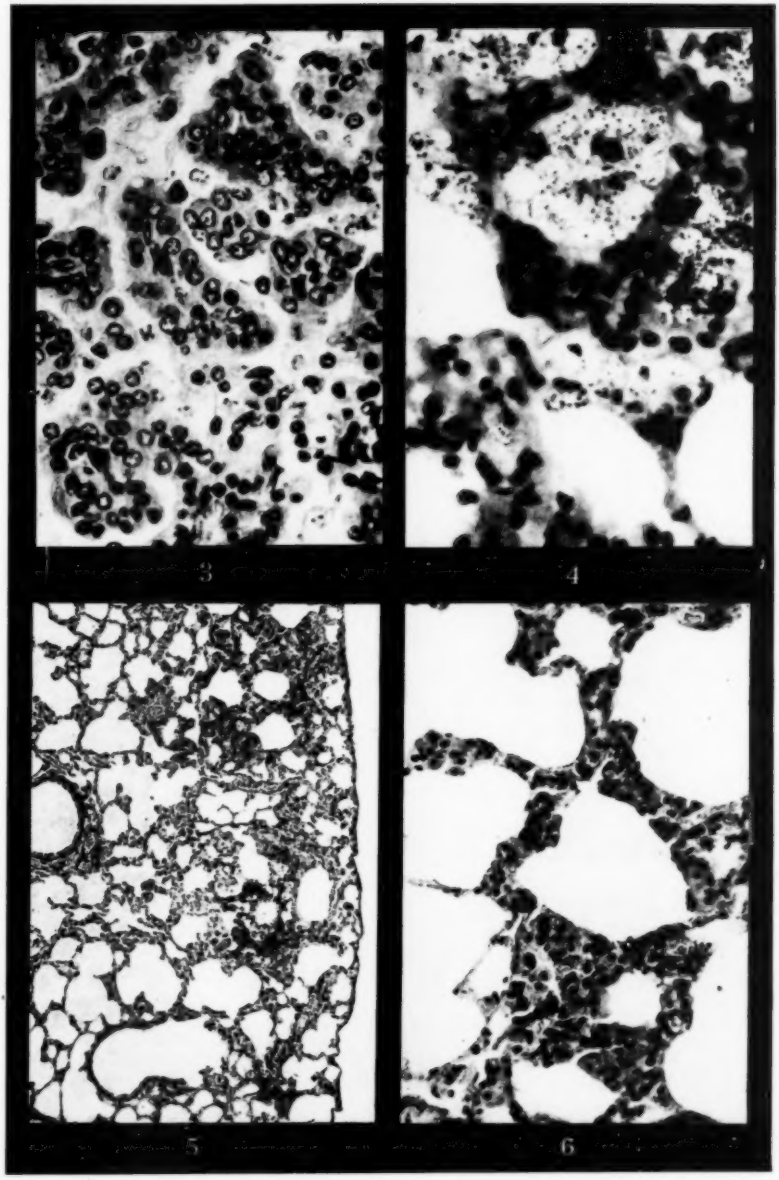

Selbie and Thackray.

some of the cells showed ill-defined vacuoles or even appeared foamy. Within the tumour mass were also variable numbers of macrophages, which frequently appeared as large foamy cells, or took the form of dust cells with their cytoplasm thickly set with black particles. When free in the cleft-like spaces or in their fully developed forms their nature was obvious, but when cells of this series with inconspicuous cytoplasmic inclusions were intimately mixed with the tumour cells in the more solid parts of the growth, there was sometimes some difficulty in distinguishing them. The fully developed foam and dust cells are easily distinguished from the tumour cells in Fig. 4.

The tumour cells were supported by a scanty connective-tissue stroma in which vessels were few, though occasionally a single large blood vessel could be seen traversing the tumour. The cleft-like spaces within the tumours usually contained varying numbers of dust cells, foam cells, and sometimes detached degenerate tumour cells. None of the tumours showed any suggestion of invasiveness, and no metastases in lymph nodes were observed.

The tumours were remarkably similar to one another; such variations as there were concerned the number of lymphocytes present and the prominence of the spaces. In some tumours the stroma was heavily infiltrated with lymphocytes, so compressing the central cleft-like spaces that at first sight the tumour appeared to be solid. A similar uniformly solid effect was apparent in some other tumours in which these spaces were tightly packed with dust cells or foam cells, particularly where the tumour cells themselves were predominantly foamy.

Apart from the more obvious circumscribed tumours there were numerous areas in the lungs, most of them just beneath the pleural surface and clear of the bronchiolar endings, where swelling of the alveolar epithelium could be seen (Fig. 5). In these areas the enlarged epithelial cells first formed a continuous cubical lining to the alveoli, while later proliferation resulted in rounded infoldings into the alveolar space with epithelial cell layers more than one cell thick, these infoldings finally developing into papillary masses almost filling the alveolus. Such areas were taken to represent the earliest stages of tumour formation (Fig. 6).

DISCUSSION.

The natural incidence of pulmonary tumours is undoubtedly low in CBA mice of an age comparable to those used in our experiments, which were all less than 11 months old when they were killed. In our 51 untreated mice we found only one tumour, while Gorer (1940) found none in 51 CBA mice less than 14 months old, and Cowen (1947) stated that CBA mice rarely have any at 1 year of age. In older CBA mice, however, pulmonary tumours are more frequent, as has been shown by Gorer (1940), who found 4 with tumours in 29 males over 14 months old, an incidence of 14 per cent, but none in a similar group of 38 females, giving an overall incidence of 6 per cent. It is thus evident from the results of our experiments that urethane, given by injection or by mouth, is highly potent in accelerating the appearance of lung adenomas in CBA mice. The relatively low yield of tumours after intranasal instillation could be attributed to the small amount of urethane absorbed, although it would appear from the results obtained by Orr (1947) that intranasal methylcholanthrene may act locally in producing pulmonary tumours. Our figures, like those of other workers,

show no significant difference in incidence between males and females (Table I), but Larsen and Heston (1945) have found that urethane administered to strain A and strain C mice on a dose/weight basis incites the formation of approximately 50 per cent more tumours in male than in female mice.

There are marked differences of opinion regarding the origin of these tumours While some regard the epithelium of the terminal bronchioles as the source of the tumours, believing it to proliferate and permeate the lung peripherally, others picture the alveolar lining cells, normally insignificant, increasing in size and numbers, to form a tumour-like mass as the change progressively affects a circumscribed group of alveoli. From the examination of our material it seems highly improbable that the tumours could have owed their origin to a proliferation of bronchiolar epithelium in view of the typical situation of the tumours at the periphery of the lung, and also in view of the absence of any apparent overgrowth of the bronchial and bronchiolar epithelium itself, such as is seen in infection with grey lung virus (Andrewes and Glover, 1945). Further, we have followed selected tumours through serial sections, and have demonstrated a definite gap between the tumour mass and the unproliferated end of the nearest bronchiole, an observation previously made, among others, by Tyzzer (1907), and by McDonald and Woodhouse (1942). Clearly the size of the tumours is such that many of them must extend around terminal bronchioles and acquire a secondary connection with them, but undue importance should not be attached to this chance association. The fact that some tumours are definitely independent of the terminal bronchioles is surely of greater significance than that some of the tumours have come to have an apparent connection.

The appearances of the early stages in the development of the tumours, where proliferation of the alveolar lining occurs *in situ*, lend further support to an alveolar origin for these tumours. Various opinions have been held in the past as to the nature, and even existence of an alveolar lining ; our studies support the conclusions of Ross (1939), who recognized an epithelial lining, not visible in ordinary histological preparations in its resting state, but becoming apparent as a continuous cubical lining in response to certain stimuli. The epithelial cells of this lining he distinguished from the phagocytic cells also present in the alveolar walls. We regard this alveolar epithelium as the tissue of origin of the tumours, the alternative, that the growths are reticulo-endothelial in nature, having little or nothing to recommend it.

A further point in dispute concerns the aetiology of these growths. Urethane is rapidly diffusible, and is therefore unlikely to act as a carcinogenic agent. It has long been known that urethane, a mitotic poison, is specially active against leucocytes, causing a reduction in the number of lymphocytes in the blood stream, as described by Hawkins and Murphy (1925) and confirmed by Dustin (1947). It is thus probable that the drug reduces the body's powers of resistance to some tumour-stimulating influence already present, possibly a virus. That there is such a lowering of resistance is borne out by the considerable mortality among the mice during the experiment ; in the survivors, too, areas of pneumonia were often seen, usually of the monocytic type noted by Nettleship, Henshaw and Meyer (1943), but like them, we were impressed by the dissociation of the tumours and the pneumonic areas. Indeed, the tumours seemed remarkably free from evidence of past or present inflammation, as was noted by Tyzzer (1909). The association of inflammatory collapse with tumours noted by Orr (1947) may have

been accounted for by the configuration of the tumours rendering them prone to terminal inflammatory involvement, as he worked largely with mice dead from natural causes.

In conclusion, our interpretation of the histological appearances supports the original suggestion of Nettleship, Henshaw and Meyer (1943) that the tumours arise from alveolar epithelium and are possibly a result of stimulation by a virus, resistance to which is diminished by the administration of urethane.

SUMMARY.

The repeated administration of urethane incited the formation of lung adenomas in all of 24 CBA mice receiving the drug by intraperitoneal injection, in all of 9 CBA mice after oral administration, and in 4 of 20 CBA mice which were given the drug by intranasal instillation, whereas the incidence of such tumours was only 1 in a comparable group of 51 CBA mice.

From histological examination of the lung adenomas in these mice and of early stages of tumour formation, it is concluded that the tumours arise from alveolar epithelium and are not preceded by pneumonic changes.

It is suggested that urethane acts by lowering resistance to some tumour-stimulating influence, possibly a virus, already present in the host.

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INFLUENCE OF TESTOSTERONE PROPIONATE ON THE INDUCTION OF SUBCUTANEOUS TUMOURS IN MICE BY 20-METHYLCHOLANTHRENE.

J. FLAKS.*

From the Department of Experimental Pathology and Cancer Research, University of Leeds.

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A great deal of experimental work has been carried out during the past decade on the influence of androgens on tumours. It was shown (Flaks and Ber, 1938*a*, 1938*b*, 1939) that large doses of testosterone propionate delayed or inhibited the appearance of skin cancer in mice painted with a 0·3 per cent benzene solution of methylcholanthrene or benzpyrene. Since these results were published, testosterone propionate has been shown to exert an inhibitory influence on the development of spontaneous mammary tumours in mice (Nathanson and Andervont, 1939; Lacassagne and Raynaud, 1939; Loeser, 1941; Jones, 1941; Heiman, 1944).

The present experiments were undertaken in order to investigate the influence of testosterone propionate on subcutaneous tumours.

MATERIAL AND METHODS.

Two hundred and forty-three mice were employed in 8 experiments, in which varying amounts of the carcinogenic agent and androgen were used. The number of mice in the experiments had to be limited, owing to the extremely large doses of testosterone propionate administered. An equal number of mice served as control in each experiment. Two to four months old mice of mixed stock (30 females), CBA low cancer strain (110 males and 70 females), C_3H (13 males) and Strong A (20 females) high cancer strains, were employed. The CBA mice were the progeny of females suckled by an RIII mouse 5 to 9 generations previously. All the females were virgins. Five mice were kept in one box and fed on rat-cake, wheat, bread and water *ad libitum*, with an addition of cod liver oil once a week.

Administration of 20-methylcholanthrene.

On the first day of the experiment all animals received the carcinogenic agent: 77 control and 76 experimental mice by injection, and 45 control and 45 experimental by implantation of pellets. 0·125 mg., 0·25 mg. and 1·0 mg. of methylcholanthrene in 0·1–0·25 c.c. of olive oil were injected subcutaneously near the middle line of the back. The pellets consisted of 5 per cent methylcholanthrene in cholesterol (Shear, 1936; Andervont and Shear, 1942), and contained 1·0 mg. of the carcinogen. The diameter of the pellets was 3 mm., the length 7 mm., and weight about 20 mg. The pellets were implanted under ether anaes-

* Bertram Parkinson Research Fellow.

thesia with a glass trocar. The trocar was introduced under the skin just above the root of the tail and advanced to the middle of the back, where the pellets were deposited. The small wound was closed with a single metal clip. In experiment 5 the pellets were left permanently under the skin ; in experiment 6 they were removed after 8 weeks, and after 4–5 weeks in experiment 7. To remove the pellet a skin incision was made about 1 cm. from the pellet. In a number of cases the removal was easy, but in others a capsule had already formed around the pellets, and this had to be removed either partially or completely.

Administration of testosterone propionate.

Ninety-five mice received weekly injections of 0·25 c.c. of a 2·5 per cent solution of testosterone propionate in arachis oil over a period of 10 to 12 weeks. The first injection was given simultaneously with methylcholanthrene except in experiment 8, in which the injections started 2 weeks previous to the administration of the carcinogen. The total dose was 50·0 to 75·0 mg. in 2·5 to 3·0 c.c. of arachis oil. The injections were made under the skin of the abdomen, and were remote from those of the carcinogenic agent which was given on the back. The first injections were made in the region of both groins until there was no more space available, while successive injections were given at different points over the whole abdominal area. It was difficult to introduce 2·5 to 3·0 c.c. of the oily solution during the 10 to 12 weeks without losing a part of the solution. The loss of testosterone propionate was minimized by advancing the needle to the opposite side of the abdomen before injecting.

Twenty-six mice were implanted with 2 to 4 tablets of testosterone propionate under the skin of the abdomen on the day of administration of the carcinogenic agent. The tablets were implanted by means of a trocar in the same manner as the methylcholanthrene-cholesterol pellets.

Control mice.

In the experiments in which the mice were injected with testosterone propionate the controls were given pure arachis oil in amounts equal to those containing the testosterone propionate and at the same sites and intervals. In the experiments in which the mice received tablets of testosterone the controls were given no control tablets.

The appearance of tumours was recorded at weekly intervals. Palpation of the skin in the region of the injection or implantation of the carcinogenic agent was helpful in detecting the earliest tumours, which were preceded by some thickening and lack of mobility of the skin in this area. Only nodules which grew during the succeeding period of observation were recorded as tumours. Mice bearing tumours were kept until they died. Sixty of the induced tumours were fixed in 10 per cent formalin for histological examination.

RESULTS.

A. *Mice injected with methylcholanthrene.*

Experiment 1 (1·0 mg. of methylcholanthrene, 62·5 mg. of testosterone propionate ; 10 control and 10 treated mice):—The first tumour appeared at 10 weeks in the control and at 15 weeks from the beginning of the experiment in the

treated mice (Table I). At the end of 14 weeks 8 tumours were present in the control, and none in the treated group. The last two control mice died with tumours 19 weeks from the start of the experiment; two of the treated mice

TABLE I.—*Experiment* 1: *Development of Subcutaneous Tumours in CBA Mice Injected with* 1 *mg. of* 20*-methylcholanthrene in Olive Oil. Ten Treated Mice Received* 62·5 *mg. of Testosterone Propionate during the First* 10 *Weeks of the Experiment.*

Week.	10 control mice.				10 treated mice.				Total tumours.	
	Living.		Dead.		Living.		Dead.			
	Without tumour.	With tumour.	Without tumour.	With tumour.	Without tumour.	With tumour.	Without tumour.	With tumour.	Control.	Treated.
8	10	0	0	0	10	0	0	0	0	0
9	10	0	0	0	10	0	0	0	0	0
10	9	1	0	0	10	0	0	0	1	0
11	5	5	0	0	10	0	0	0	5	0
12	5	5	0	0	10	0	0	0	5	0
13	3	7	0	0	10	0	0	0	7	0
14	2	8	0	0	10	0	0	0	8	0
15	0	9	0	1	7	3	0	0	10	3
16	0	7	0	3	5	5	0	0	..	5
17	0	4	0	6	3	6	0	1	..	7
18	0	2	0	8	3	6	0	1	..	7
19	0	0	0	10	3	5	0	2	..	7
20	..	..	..	..	1	5	0	4	..	9
21	..	..	..	..	0	6	0	4	..	10
22	..	..	..	..	0	3	0	7	..	..
23	..	..	..	..	0	3	0	7	..	..
24	..	..	..	..	0	2	0	8	..	..
25	..	..	..	..	0	2	0	8	..	..
26	..	..	..	..	0	0	0	10	..	..

with tumours survived for 26 weeks. The average latent period was 12 weeks in the control and 17 weeks in the treated group. The average time of survival from the beginning of the experiment was 16 and 21 weeks respectively. During 10 weeks of treatment the average increase in weight of the control mice was 5 g. and of the treated mice 6 g.

TABLE II.—*Summary of Experiments* 1 *to* 7 *on the Influe*

Experiment.	Strain and sex.		Total mice.	Methylcholanthrene. Injected. Conc.	Injected. Mg.	Implanted. Conc.	Implanted. Mg.	Testosterone propionate. Inj. mg.	Impl. mg.	Tum Numerator = mice with t															ths withou ominator			
										8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21		24.	2
1	CBA	c.	10	0·4%	1·0	—	—	62·5	—	..	..	1/0	4/0	..	2/0	1/0	2/0	..	..	..	..	..	..		..	..
	♀+♂	tr.	10							..	..	..	..	..	..	..	3/0	2/0	2/0	..	..	2/0	1		..	..
2	CBA	c.	20	0·1%	0·25	—	—	—	75·0‡	0/1	2/0	..	..	4/0	1/0	..	0/2	1/0	3/0	2/0	1/0	..	1		..	..
	♂	tr.	20							..	0/1	..	..	..	1/0	1/1	0/2	1/0	5/0	1/0	..	..	..		..	..
3	C_3H	c.	7	0·1%	0·25	—	—	—	60·0	1/0	0/1	1/1	1/0	1/0	..	..	..	..	..	1/0	..	..	..		..	..
	♂	tr.	6							..	..	..	..	..	..	0/1	..	1/0	1/1	..	..	..	0		..	..
4	CBA	c.	10	0·05%	0·125	—	—	75·0	—	..	1/0	..	..	..	1/0	1/0	1/0	..	..	..	..	..	..		1/0	..
	♂	tr.	10							..	..	..	..	0/5	..	..	..	1/0	..	..	1/0	..	..		..	..
5*	Stock	c.	15	—	—	5%	1·0	75·0	—	..	..	..	..	..	..	2/0	2/0	1/0	..	1/0	..	2/0	1		1/0	..
	♀	tr.	15							..	..	..	..	..	..	..	0/1	0/1	2/0	3/0	1/1	..	1		1/0	..
6†	A	c.	10	—	—	5%	1·0	—	40·0	..	..	0/1	1/0	2/0	0/1	..	..	..	1/0	..	1/0	..	..		..	..
	♀	tr.	10							..	..	..	..	0/1	0/1	1/1	..	1/0	..	1/0	..	1/0	..		..	..
7‡	CBA	c.	20	—	—	5%	1·0	75·0	—	..	..	..	..	..	..	3/0	1/0	..	..	..	..	..	..		1/0	..
	♀+♂	tr.	20							..	..	..	..	0/4	..	2/0	2/0	1/0	1/0	1/0	..	..	..		..	..

Abbreviations employed: c. = = treated;

* The implanted hydrocarbon-cholesterol pellets manently. to 5 weeks. ‡ Mice dying before the first tumour excluded. with 75·0 mg. of testosterone propionate.

Experiment 2 (0·25 mg. of methylcholanthrene, 75·0 mg. of testosterone propionate; 20 control and 20 treated mice):—The first tumour appeared in the control mice after 9 weeks, and in the treated after 13 weeks (Table II). At the end of 12 weeks 6 tumours were present in the control and none in the treated group. After 15 weeks 3 control and 4 treated mice died without tumours. After 24 weeks 15 tumours developed in the control and 10 tumours in the treated mice.

Experiment 3 (0·25 mg. of methylcholanthrene, 60·0 mg. of testosterone propionate; 7 control and 6 treated mice):—The first tumour was observed in the control after 8 weeks, and after 16 weeks in the treated mice (Table II). Two control mice died without tumours early in the experiment. At the end of 15 weeks 4 tumours were present in the control and none in the treated group. After 20 weeks all control mice died with tumours, while 2 tumours developed in the treated group. The 6 treated mice died within 22 weeks, 2 of them with tumours.

Experiment 4 (0·025 mg. of methylcholanthrene, 75·0 mg. of testosterone propionate; 10 control and 10 treated mice):—During the 12th week of the experiment 5 mice suddenly died in the treated group. The first tumour appeared after 9 weeks in the control as compared with 16 weeks in the treated mice (Table II). After 14 weeks 3 control mice developed tumours, while none appeared in the treated mice. After 28 weeks 6 tumours occurred in the control mice and 3 tumours in the treated mice.

Experiment 8 (1·0 mg. of methylcholanthrene, 50·0 mg. of testosterone propionate; 30 control and 30 treated mice, 15 of each sex):—Injections of testosterone propionate were started 2 weeks before the administration of the carcinogen. All the mice were killed after 13 weeks and their tumours recorded. In cases where small nodules were present their nature was established by histological examination.

It is shown in Table IV that after 13 weeks 9 tumours were present in the 14 surviving control females (64 per cent), while an equal number of treated

tosterone Propionate on the Induction of Subcutaneous Tumours.

hs without tumours in weeks.
minator = mice dying without tumours.

24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.	38.	39.	40.	41.	42.	43.	44.	Survivors without tumours after 44 weeks.	Effective total mice.‡	Total tumours.	Minimal latent time—weeks.	50% latent time —weeks.
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	10	10	10	10·8
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	10	10	15	15·4
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	19	15	9	11·6
..	..	..	..	..	..	..	1/0	..	..	1/0	..	..	..	..	..	..	..	..	1/0	..	3	19	13	13	15·7
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	7	5	8	9·4
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	5	2	16	16·0
1/0	..	..	..	..	1/0	..	..	..	..	..	..	1/0	1/0	..	..	..	..	1/0	..	..	0	10	10	9	14·6
..	..	..	..	1/0	1/0	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	5	4	16	17·5
1/0	..	1/0	..	..	..	0/1	..	..	..	1/0	..	0/1	..	..	..	..	..	1/0	..	..	0	15	15	14	16·2
1/0	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	13	10	17	17·6
..	..	..	..	..	..	..	1/0	..	..	..	..	..	..	..	..	0/1	..	..	..	..	0	9	7	11	12·4
..	..	..	..	..	..	..	0/1	..	..	..	..	..	..	..	..	..	..	..	..	..	2	8	4	14	15·0
1/0	..	..	1/0	..	..	..	..	..	..	..	1/0	..	..	..	..	..	..	..	0/3	0/1	7	20	9	14	15·2
..	..	1/0	..	1/0	0/2	..	..	..	..	..	..	..	0/1	0/2	..	..	..	..	..	..	1	16	10	14	14·8

= treated; conc. = concentration.

manently. † Removed after 8 weeks. ‡ Removed after 4
excluded. ‖ Ten mice were implanted and 10 injected

females developed only 3 tumours (21 per cent). The male mice did not show a clear-cut result. Five treated males died early without tumours. The surviving 10 males showed 4 tumours, while 15 control males developed 7 tumours. This difference is not statistically significant.

The total number of tumours in males and females was 16 out of 29 control mice (55·1 per cent) and 7 out of 24 treated mice (29·1 per cent)—a statistically significant difference.

B. *Mice implanted with methylcholanthrene-cholesterol pellets.*

Experiment 5 (1·0 mg. of methylcholanthrene, pellets left permanently; 75·0 mg. of testosterone propionate; 15 control and 15 treated mice):—The first tumour appeared after 14 weeks in the control and after 17 weeks in the treated mice. Three treated mice died without tumours between the 15th and 19th week (Table II). At the end of 16 weeks 5 tumours were present in the control and none in the treated group. After 32 weeks 14 control mice and 9 treated mice developed tumours. The average increase in weight of testosterone-treated mice during the first 8 weeks of treatment was the same as in the control mice. At the end of 15 weeks, however, a slight decrease in both control and treated mice occurred.

Experiment 6 (1·0 mg. of methylcholanthrene, pellets removed after 8 weeks; 40·0 mg. of testosterone propionate; 10 control and 10 treated mice):—The first tumour arose after 11 weeks in the control, and after 14 weeks in the treated mice (Table II). At the end of 13 weeks 3 tumours were found in the control and none in the treated group. Two control and 3 treated mice died early without tumours. After 36 weeks there were 7 tumours in the control and 4 tumours in the treated group.

Experiment 7 (1·0 mg. of methylcholanthrene, pellets removed after 4–5 weeks; 62·5–75·0 mg. of testosterone propionate; 20 control and 20 treated mice):—In both groups the first tumour developed after 14 weeks (Table II). At the end of 20 weeks 4 tumours were present in the control and 7 tumours in the treated mice. After 28 weeks 8 tumours occurred in the control, while 10 tumours were present in the treated group. This result differs from the results of all the other experiments (see p. 391).

The injection of testosterone propionate over a period of 10 to 12 weeks (Experiments 1, 2 and 4) or the implantation of approximately the same amount of the hormone (Experiments 2 and 3) delayed the appearance of subcutaneous tumours in mice receiving the carcinogen by about 4–5 weeks. No effect of testosterone propionate was observed in experiments in which the carcinogen was implanted in pellets and later removed.

The amount of hormone absorbed from the injected oily solution is not known. The amount absorbed from the implanted tablets was calculated from the initial weight of the tablets and the weight after removal on the death of the host. The rate of absorption was about 4 mg. per month. In mice surviving for more than 5 months the tablets were almost completely absorbed.

Sixty tumours were examined histologically. Fifty-six were sarcomata (93·3 per cent) and 4 skin carcinomata (6·7 per cent).

DISCUSSION.

In Experiment 1, in which 1·0 mg. of the carcinogenic agent was employed, all the control and treated mice died with tumours. This shows that testosterone had only a delaying influence, and was unable to prevent the carcinogenic action of methylcholanthrene.

In Experiments 2 to 4, in which smaller doses of methylcholanthrene were injected (0·25 mg. and 0·125 mg.), testosterone propionate caused not only delay in tumour appearance, but also a lower tumour incidence after 24 weeks. Twenty-six tumours developed in 36 control mice (72·2 per cent) and 14 tumours in 29 treated mice (48·2 per cent)—a statistically significant result.

In Experiments 5 to 7, 1·0 mg. of methylcholanthrene was implanted in 5 per cent hydrocarbon-cholesterol pellets. This method was at first considered to be more precise, because it was thought that the carcinogenic agent would be absorbed in uniform amounts over a given period. However, a comparison of the tumour incidence after injections and pellet implantations showed that the tumours did not occur earlier or at more regular intervals with the latter method.

In Experiment 6 the pellets were removed after 8 weeks, and in Experiment 7 after 4 to 5 weeks. In some mice the pellets were encapsulated in varying degrees, and the removal of the capsule in a number of mice could not be avoided. This is likely to have influenced the tumour incidence, and thus to have made a comparison between control and treated mice unreliable. Therefore, the results of Experiments 6 and 7 may be open to criticism. In these experiments no effect of testosterone propionate was observed. This was in contrast with the experiments in which methylcholanthrene was injected in olive oil. No adequate explanation can be given at present. However, the findings of the experiments on the induction of sarcomata in mice implanted with methylcholanthrene-cholesterol pellets by Shimkin and Wyman (1947) may be of interest. They implanted 0·002 to 0·5 mg. of methylcholanthrene in a 0·1 to 25 per cent concentration in cholesterol into C_3H male mice. No shortening of the latent period was observed when higher concentrations and doses were implanted. The authors suppose that the localization of the hydrocarbon by means of cholesterol may have removed the differences in the latent period, as compared with that of the mice given the carcinogen in tricaprylin.

In the present experiments, in which 5 per cent hydrocarbon-cholesterol pellets were implanted and remained permanently under the skin, the latent period and the tumour incidence were similar to those in which mice were injected with the carcinogenic agent in much lower concentrations (0·4 to 0·1 per cent). However, when the pellets were removed after a short exposure (4 to 5 weeks) the latent period was longer and the incidence of tumours lower than in mice injected with lower concentrations of the carcinogen. This may have altered the results of testosterone treatment, because the bulk of the androgen was injected long before the tumours began to develop. The unequal removal of the carcinogenic agent in individual control and treated mice may also have been responsible for the negative result, because mice treated with testosterone were not discarded, even if it was felt that a small part of the carcinogenic pellet may have been left.

The need for larger groups of animals in experiments in which the influence on the latent period or on the incidence of tumours is investigated was fully

realized. In the present experiments the large doses of testosterone propionate employed (about 8·0 g. of the hormone in all) precluded the use of larger groups of animals. Owing to the rather small number of mice employed in Experiments 1 to 7 the number of tumours induced in the control and experimental mice at 4-weekly intervals in each of the experiments was added and the tumour incidence compared (Table III). This was considered admissible, as the dose of the carcinogen was the same in both groups of each experiment and the dose of testosterone propionate was almost uniform (62·5 to 75·0 mg.).

TABLE III.—*Experiments* 1 *to* 7 : *Total Incidence of Tumours.*

Weeks.	Control.			Treated.		
	Effective‡ total mice.	Number of tumours.	Per cent of tumours.	Effective‡ total mice.	Number of tumours.	Per cent of tumours.
12	90	19	21·1	80	0	0
16	90	38	42·2*	76	17	22·3*
20	90	50	55·5†	76	39	51·3†

* This difference is statistically significant.
† This difference is not significant.
‡ Mice dying before the first tumour occurred are excluded.

The following results were obtained when the numbers of tumours in the control and treated mice occurring at 4-weekly intervals were compared. Up to the end of 12 weeks from the beginning of the experiments the results were clear-cut. There were no tumours in the 80 treated mice, and 19 tumours were observed in 88 control mice alive at that time. Between the 12th and 16th week the tumours began to appear in the treated mice. At the end of this period 38 tumours were found in the control (42·2 per cent) as compared with 17 tumours in the treated mice (22·3 per cent). Thus, although there were twice as many tumours in the control as in the experimental mice, approximately the same number of new tumours developed in both groups during these 4 weeks. Between the 16th and 20th week 12 new tumours appeared in the control and 22 in the treated mice. The total number of tumours reached 50 in the control and 39 in the treated mice.

A significant difference in the influence of testosterone propionate on the development of tumours in males and females was observed in Experiment 8 (Table IV).

TABLE IV.—*Experiment* 8 : *Incidence of Tumours after* 13 *Weeks.*

Sex.		Number of mice.	Effective† total mice.	Number of tumours and deaths without tumours.*	Per cent of tumours.
♀	Control	15	14	9/1	64
	Treated	15	14	3/1	21
♂	Control	15	15	7/0	46
	Treated	15	10	4/5	40

* Numerator = mice with tumours. Denominator = mice dead without tumours.
† Mice dying before the first tumour occurred are excluded.

The massive doses of testosterone propionate employed did not cause any loss of weight in the treated mice. On the contrary, a slight increase in weight of the treated in comparison with the control mice was observed. Testosterone propionate exerted a marked influence on male and female genital organs :

hypertrophy of the seminal vesicles in the male, enlargement of the uterus and vagina, hypertrophy of the clitoris and an extreme hypertrophy of the clitoral glands in the female. Atrophy of the thymus and adrenals was the most striking finding in both sexes. Enlargement of the kidneys was also encountered. In addition, marked pigmentation of the mammary nipples in all dark-coated females (Flaks, 1948) and prolapse of the vagina and uterus in a number of mice was observed.

No connection could be found, at the present stage of the experiments, between the delaying influence of testosterone on carcinogenesis and the well-defined changes induced by the hormone in many organs. The hyperpigmentation of the nipples, which seems to be an outcome of increased activity of oxidation processes induced by testosterone propionate in the tissues, and indicated by the formation of black melanin, may prove to be of help in the understanding of the effect of testosterone on tumours.

The mechanism of the interference of testosterone with the action of methylcholanthrene is not known. Some workers consider the inhibitory influence of testosterone on spontaneous mammary tumours in mice as a castration effect. According to these workers, testosterone, by inhibiting the production of oestrogen in the ovaries inhibits the development of the mammary glands. They claim that this effect is similar to that first obtained by Lathrop and Loeb (1916), and Cori (1927), who showed that castration of female mice, at an early age, inhibited the development of breast cancer. The present experiments, in which an inhibitory influence on subcutaneous tumours induced by methylcholanthrene was only obtained after large doses of testosterone, suggest that this influence may not be hormonal. Doses which gave hormonal effects, such as masculinization, were not sufficient to produce even slight inhibition of tumours (Flaks and Ber, 1938*a*, 1938*b*). It is possible, therefore, that testosterone may directly interfere, at least temporarily, with the malignant transformation of the normal cell under the influence of methylcholanthrene.

SUMMARY.

1. The influence of large doses of testosterone propionate (50·0–75·0 mg.) on the latent period and the incidence of subcutaneous tumours induced by 20-methylcholanthrene in 121 mice of several strains was investigated. One hundred and twenty-two mice served as controls.

2. An average delay of about 4 weeks in the appearance of 50 per cent of subcutaneous tumours induced by methylcholanthrene was observed after 62·5–75·0 mg. of testosterone propionate injected over a period of 10–12 weeks.

3. This result confirms and extends the findings of previous experiments in which the influence of testosterone propionate on skin cancer induced by painting with methylcholanthrene was studied.

4. The total incidence of tumours in mice treated with testosterone propionate was significantly reduced only up to the 16th week after the administration of the carcinogen (42·2 per cent to 22·3 per cent). No significant difference was observed later.

5. Implantation of testosterone propionate exerted a similar influence.

6. There was no decrease in weight of mice after large doses of testosterone propionate.

7. Testosterone propionate produced masculinization and hyperpigmentation of the mammary nipples in female mice. A few mice developed prolapse of the vagina and uterus.

8. The nature of the delaying influence of testosterone propionate on tumour induction remains to be elucidated.

9. Histological examination of 60 of the induced tumours revealed 56 sarcomata (93·3 per cent) and 4 squamous-celled carcinomata (6·7 per cent).

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THE INHIBITORY INFLUENCE OF 20-METHYLCHOLANTHRENE ON THE ACTION OF CHORIONIC GONADOTROPHIN ON THE OVARIES OF IMMATURE MICE.

J. FLAKS.*

From the Department of Experimental Pathology and Cancer Research, University of Leeds.

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CARCINOGENIC hydrocarbons have been shown to inhibit the growth of transplantable tumours in mice and rats, and also of spontaneous breast tumours in mice (Haddow, 1935, 1938*a*, 1938*b*, 1938*c*; Haddow and Robinson, 1937). This tumour-inhibitory influence of carcinogenic hydrocarbons was found to be non-specific, and was shown to affect the growth of the whole body of young animals (Haddow, Scott and Scott, 1937). It was also found to depend on a low protein content of the diet (Elson and Warren, 1947; Elson and Haddow, 1947). No inhibition of the growth-promoting action of anterior pituitary growth hormone was observed in rats treated with 1:2:5:6-dibenzanthracene (Lees, 1937*a*, 1937*b*, 1937*c*), but a lowering of fertility in treated rats has been noted (Haddow and Robinson, 1937).

The present experiments were undertaken in order to investigate the influence of 20-methylcholanthrene on the action of gonadotrophic hormone on the ovaries of immature mice, following the injection of chorionic gonadotrophin. All stages of the reaction, i.e. the growth of the follicles, bleeding into their lumina, and luteinization have been examined.

MATERIAL AND METHODS.

The gonadotrophic reaction was carried out in a manner similar to the typical Aschheim-Zondek pregnancy test (1928*a*, 1928*b*). A commercial preparation of chorionic gonadotrophin and mice of inbred strains were used. One ampoule of "Pregnyl-Organon" containing 100 I.U. of chorionic gonadotrophin was dissolved in 8 c.c. of distilled water. Each mouse received 0·6 c.c. of the solution, e.g. 7·5 I.U. (follicle stimulating and luteinizing hormone). Immature CBA, comprising a number of mice which were the progeny of females suckled by an RIII mouse 9–10 generations previously, and Strong A female mice, were injected with 7·5 I.U. of chorionic gonadotrophin. While in CBA mice, following the injection of chorionic gonadotrophin, blood-filled follicles (blood points) were almost invariably observed, in Strong A mice they were only occasionally seen (Tables I and II) For choice, mice of the CBA strain are, therefore, to be preferred as the test animals.

Litters of immature female mice of both strains, 3 to 4 weeks old and weighing not less than 7 g. and not more than 8·5 g., were divided as far as possible into

* Bertram Parkinson Research Fellow.

two groups. The experimental mice received either one to three subcutaneous injections of 1·0–2·0 mg. of methylcholanthrene (1 per cent solution in olive oil) at intervals of 3 to 4 days, or a single injection of a similar dose intraperitoneally. The control mice were injected at the same time with the same quantity of pure olive oil. Injections of the gonadotrophic hormone started on the day following the last injection of the carcinogen, i.e. 4 to 8 days after the first injection of the methylcholanthrene. Each mouse of both groups received 6 injections of 0·1 c.c. during a period of 36 hours. The injections were given 3 times during the daytime at 4-hourly intervals, giving a total of 7·5 I.U. of chorionic gonadotrophin. Seventy-two and 96 hours after the first injection of the hormone vaginal smears were made. After 100 hours all the mice were killed, weighed, and their ovaries inspected with a hand lens for the presence of blood points and corpora lutea. Microscopical examination of the ovaries compressed in a drop of glycerol between two slides was helpful in detecting small luteinized follicles. In cases of doubtful reactions the ovaries were examined histologically by means of serial sections.

RESULTS.

Control CBA mice, injected with olive oil and chorionic gonadotrophin, showed, after 96 hours, a typical oestrous or metoestrous reaction of the vagina and enlargement of the uterus. One or more blood points were generally found in one or both ovaries as well as numerous well-developed corpora lutea. Experimental CBA mice, which received one or more subcutaneous injections of methylcholanthrene previous to chorionic gonadotrophin, did not show blood points and only very few abortive corpora lutea (Table I). This difference between control and methylcholanthrene-treated CBA mice varied according to the dose injected and could easily be observed macroscopically, while histological examination was required to ascertain the degree of follicle maturation. The oestrous reaction of the vaginal epithelium was usually present or only slightly inhibited (mucification of the epithelium instead of keratinization). This indicated that the initial ovarian changes leading to oestrogen production were not prevented. The growth of follicles in the carcinogen-treated mice was not always inhibited and mitoses were frequent, but no bleeding occurred into their lumina. Therefore, the macroscopic appearance of the ovaries of carcinogen and gonadotrophin-treated mice was similar to that of normal immature mice (Fig. 1–4).

The inhibition of the ovarian and vaginal reactions was more pronounced after intraperitoneal than after subcutaneous injection of the carcinogen (Tables I and II). After 8 days a loss of weight in mice which received 2·0 mg. of methyl-

EXPLANATION OF PLATE.

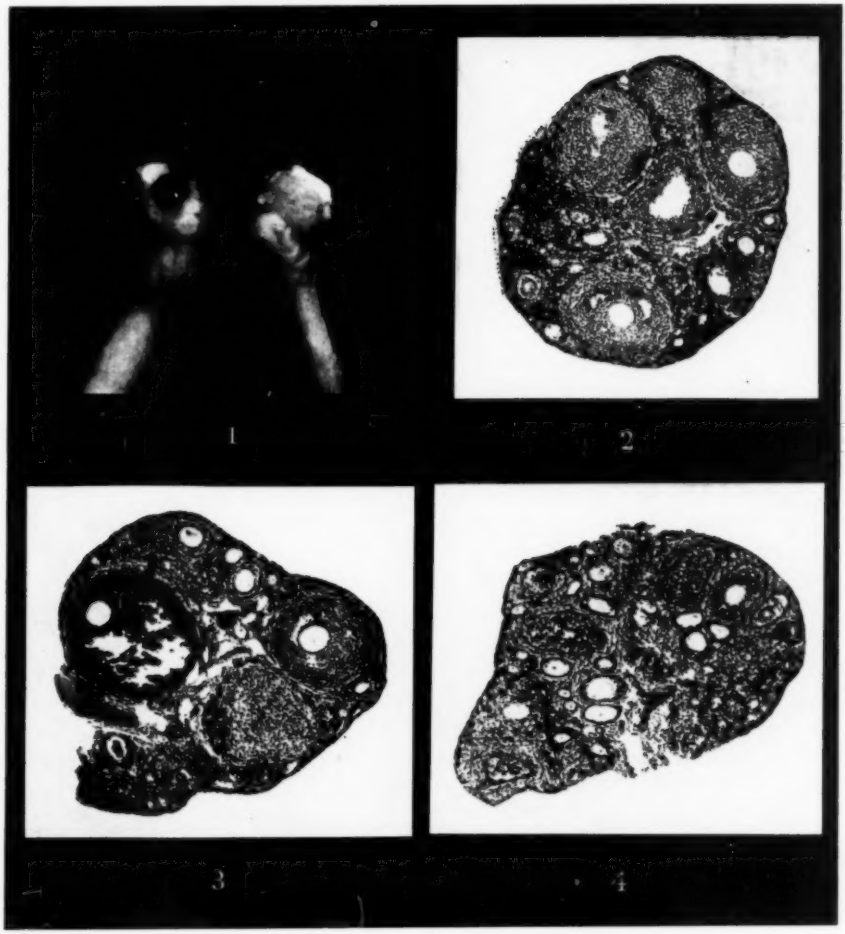
FIG. 1.—Ovaries of immature CBA mice injected with 7·5 I.U. of chorionic gonadotrophin. Left: ovary of control mouse (Table I, No. 10) with a haemorrhagic follicle and two corpora lutea. Right: ovary of mouse (Table I, No. 10 treated) injected subcutaneously with 2·0 mg. of methylcholanthrene 5 days previous to chorionic gonadotrophin. Negative reaction. × 8.

FIG. 2–4.—Ovaries of immature CBA mice. Fixed in Bouin. H.E. × 60.

FIG. 2.—Normal immature mouse.

FIG. 3.—7·5 I.U. of chorionic gonadotrophin. Haemorrhagic follicle and corpus luteum (Table I, No. 8).

FIG. 4.—7·5 I.U. of chorionic gonadotrophin 8 days after the administration of 6·0 mg. of methylcholanthrene (Table I, No. 20 treated). Negative gonadotrophic reaction.



Flaks (II).

TABLE I.—*Influence of 20-methylcholanthrene on the Gonadotrophic Reaction in CBA Immature Mice (7·5 I.U. of Chorionic Gonadotrophin).*

	Control.						Treated.					
Exp. number.	Mouse number.	Total olive oil inj. (c.c.).	Interval* (days).	Gonadotrophic reaction. I. Oe.	II. Bl.p.	III. C.l.	Mouse number.	Total m.ch. inj. (mg.).	Interval* (days).	Gonadotrophic reaction. I. Oe.	II. Bl.p.	III C.l.
Subcutaneous injections. 1	1	0·2	4	+	+	+	1	2·0	4	±	−	±
	2	,,	,,	+	+	+	2	,,	,,	±	−	±
	3	,,	,,	+	+	+	3	,,	,,	±	−	±
	4	,,	,,	+	+	+	4	,,	,,	±	−	±
	5	,,	,,	+	+	+	5	,,	,,	±	−	±
	6	,,	,,	+	+	+	6	,,	,,	±	−	±
	7	,,	,,	+	−	+	7	,,	,,	−	−	+
	8	,,	,,	+	+	+	8	,,	,,	+	−	±
	9	,,	,,	+	+	+	9	,,	,,	+	−	−
	10	,,	,,	+	+	+	10	,,	,,	−	−	−
	11	,,	,,	+	+	+	11	,,	,,	+	−	±
	12	,,	,,	+	+	+	12	,,	,,	+	−	±
	13	,,	,,	+	+	+	13	,,	,,	+	−	+
	14	,,	,,	+	+	+	14	,,	,,	+	−	+
2	15	0·4	,,	+	−	+	15	4·0	,,	−	−	−
	16	,,	,,	−	−	±	16	,,	,,	±	−	±
	17	,,	,,	+	−	+	17	,,	,,	−	−	−
	18	,,	,,	±	+	+	18	,,	,,	±	−	−
	19	,,	,,	±	+	+	19	,,	,,	±	−	−
3	20	0·6	8	+	+	+	20	6·0	8	−	−	−
	21	,,	,,		Died.		21	,,	,,	−	−	−
	22	,,	,,	−	+	±	22	,,	,,	−	−	±
	23	,,	,,	+	−	+	23	,,	,,	−	−	±
	24	,,	,,	±	+	+	24	,,	,,	−	−	−
Intraperitoneal injections. 4	25	0·1	3	+	+	+	25	1·0	3	±	−	−
	26	,,	,,	+	+	+	26	,,	,,	±	−	±
	27	,,	,,	+	+	+	27	,,	,,	±	−	±
	28	,,	,,		Died.		28	,,	,,	−	−	−
	29	,,	,,	±	+	+	29	,,	,,	−	−	−
	30	,,	,,	+	+	+	30	,,	,,	−	−	−
5	31	0·15	5	+	+	+	31	1·5	5	−	−	−
	32	,,	,,	−	−	−	32	,,	,,		Died.	
	33	,,	,,	−	−	+	33	,,	,,	−	−	−

Oe. = oestrus ; Bl.p. = blood point ; C.l. = corpora lutea ; m.ch. = 20-methylcholanthrene. + under I = full oestrus reaction in the vagina ; + under II = the presence of blood points ; + under III = normally developed corpora lutea. ± under I = mucification of the vaginal epithelium in proestrus without keratinization ; ± under III = traces of luteinized follicles. − under I = dioestrus ; − under II = the absence of blood points ; − under III = the absence of luteinization. * = interval in days between the first injection of methylcholanthrene or olive oil and the first injection of chorionic gonadotrophin.

cholanthrene intraperitoneally was observed, while the controls showed increase in weight (Table III). The abdominal cavity of mice injected intraperitoneally was found to contain a milky fluid, which was not present in the controls injected with pure olive oil.

A similar influence of methylcholanthrene on luteinization and vaginal reaction was observed in Strong A mice (Table II). As the control mice of this strain rarely develop blood points with the dose injected, it was not possible to use their absence in methylcholanthrene-treated mice as a criterion of difference.

TABLE II.—*Influence of 20-methylcholanthrene on the Gonadotrophic Reaction in Strong A Immature Mice (7·5 I.U. of Chorionic Gonadotrophin).*

	Exp. number.	Mouse number.	Control. Total olive oil inj. (c.c.).	Control. Interval* (days).	Control. Gonadotrophic reaction. I. Oe.	Control. Gonadotrophic reaction. II. Bl.p.	Control. Gonadotrophic reaction. III. C.l.	Mouse number.	Treated. Total m.ch. inj. (mg.).	Treated. Interval* (days).	Treated. Gonadotrophic reaction. I. Oe.	Treated. Gonadotrophic reaction. II. Bl.p.	Treated. Gonadotrophic reaction. III. C.l.
Subcutaneous injections.	1	1	0·2	5	+	−	+	1	2·0	5	−	−	−
		2	,,	,,	+	−	+	2	,,	,,	−	−	−
		3	,,	,,	+	+	+	3	,,	,,	+	−	+
		4	,,	,,	+	−	+	4	,,	,,	+	−	−
		5	,,	,,	+	−	+	5	,,	,,	−	−	±
	2	6	0·3	7	+	−	+	6	3·0	7	±	−	±
		7	,,	,,	+	−	+	7	,,	,,	+	−	±
		8	,,	,,	+	−	+	8	,,	,,	−	−	±
		9	,,	,,	+	+	+	9	,,	,,	−	−	−
	3	10	0·4	5	±	−	+	10	4·0	5	±	−	+
		11	,,	,,	−	−	+	11	,,	,,	−	−	±
		12	,,	,,	+	−	+	12	,,	,,	−	−	−
		13	,,	,,	−	+	+	13	,,	,,	−	−	−
		14	,,	,,	−	−	±	14	,,	,,	±	−	+
Intraperitoneal injections.	4	15	0·1	1	+	−	+	15	1·0	1	−	−	±
		16	,,	,,	−	−	+	16	,,	,,	−	−	−
		17	,,	,,	−	−	−	17	,,	,,	−	−	±
	5	18	,,	6	−	−	+	18	,,	6	−	−	−
		19	,,	,,	−	−	−	19	,,	,,	−	−	−
		20	,,	,,	±	−	+	20	,,	,,	−	−	−
		21	,,	,,	−	−	+	21	,,	,,	±	−	+
	6	22	0·2	5	−	−	+	22	2·0	5	−	−	−
		23	,,	,,	−	−	+	23	,,	,,	−	−	−
		24	,,	,,	−	−	+	24	,,	,,	−	−	−
		25	,,	,,	−	−	+	25	,,	,,	−	−	−
		26	,,	,,	−	−	+	26	,,	,,	−	−	−

Abbreviations as in Table I.

DISCUSSION.

Human chorionic gonadotrophin employed in the experiments contains a follicle-stimulating and luteinizing factor (Prolan A and B of Zondek). The response of immature ovaries to chorionic gonadotrophin consists of follicle growth, followed by bleeding into the follicles and their luteinization. There are, however, indications that chorionic gonadotrophin itself is not a follicle-stimulating factor, and that follicle growth may be the result of stimulation of the test animal's own pituitary, which produces another gonadotrophic hormone synergistic with the injected chorionic gonadotrophin (Selye and Collip, 1933). It is not known whether methylcholanthrene interferes with the production of the synergistic hormone in the hypophysis. No statement, therefore, can be made on the mechanism by which methylcholanthrene inhibits the reaction of the ovaries to gonadotrophin (directly or through the hypophysis). To elucidate this question it would be necessary to employ gonadotrophic hormone of hypo-

TABLE III.—*Examples of the Early Influence of Subcutaneous and Intraperitoneal Injection of 20-methylcholanthrene on Weight Increase in Immature Female Mice (after 8 days).*

	Strain.		Treatment on 1st day.	Mean body weight (g.). 1st day.	Mean body weight (g.). 9th day.	Percentage increase in weight.
Subcutaneous injections.	CBA	Control (8 mice)	0·2 c.c. olive oil	8·8	12·0	36·4
		Treated (8 mice)	2·0 mg. m.ch. in 0·2 c.c. olive oil	8·5	11·4	34·1
	Strong A	Control (5 mice)	0·2 c.c. olive oil	7·9	12·0	51·9
		Treated (5 mice)	2·0 mg. m.ch. in 0·2 c.c. olive oil	8·3	9·3	12·0
Intraperitoneal injections.	Strong A	Control (6 mice)	0·2 c.c. olive oil	7·3	10·8	47·9
		Treated (6 mice)	2·0 mg. m.ch. in 0·2 c.c. olive oil	7·5	7·1	− 5·3

m.ch. = 20-methylcholanthrene.

physeal origin, as this kind of hormone produces an effect even in animals deprived of the hypophysis.

In the present experiments immature mice were injected subcutaneously or intraperitoneally with a few milligrams of methylcholanthrene from 4–8 days before the gonadotrophic reaction was carried out. A direct absorption of the carcinogen into the ovary following intraperitoneal injection seems likely, and may be responsible for the more pronounced influence on the reaction as compared with the subcutaneous injections. It seems also probable that the absorbed carcinogen acts directly on the ovarian follicles, causing the described deviations in their response. A similar influence on the gonadotrophic reaction has been previously described by Flaks and Ber (1938*a*, *b* and *c*). It was shown in these experiments that immature mice, bearing a transplantable sarcoma, lost their sensitivity to chorionic gonadotrophin. These mice did not develop oestrus, blood points or corpora lutea following the injection of many thousands of mouse units of gonadotrophin. An inhibition of follicle maturation and oestrogen formation was also induced in mice injected with extracts from urine of tumour-bearing men and women (Flaks and Ber, 1939*a*, 1939*b*). It was concluded that the immature ovarian follicles are very sensitive to the influence of toxic products apparently derived from tumour tissue.

The oestrous reaction of the vagina was not always inhibited in the mice treated with methylcholanthrene, even when an inhibition of blood points and luteinization occurred. Therefore, in these mice there was no interference with the production of oestrogen, and the vaginal epithelium did not lose its sensitivity to oestrogen. This result is also in agreement with previous experiments, in which it was shown that in immature mice bearing a transplantable sarcoma the gonadotrophic response of the ovaries was inhibited, while the vaginal response to oestrone remained unaffected (Flaks, Ber and Pakszwer, 1938). The possible production

of extra-follicular oestrogen in the theca cells by the follicle-stimulating hormone may to some extent explain the positive vaginal reaction in the carcinogen-treated mice which received gonadotrophic hormone.

Large doses of carcinogenic hydrocarbons exert a general toxic action resulting in a permanent interference with the growth of the body as a whole (Haddow, Scott and Scott, 1937; Lees, 1937). Grossly visible changes in lymphoid organs, such as marked atrophy of the spleen, lymph nodes, thymus and bone marrow, have been induced in mice and rats injected with 3:4-benzpyrene (Picard and Laduron, 1934; Maisin and Coolen, 1934; Picard, 1936). A similar effect on spleen and lymph nodes was observed in the present experiments within a week of intraperitoneal injection of 1·0 mg. or subcutaneous injection of 2·0–4·0 mg. of methylcholanthrene. From a limited number of observations no influence was seen on mitosis in the membrana granulosa of the ovarian follicles. It is not known whether the atrophy of lymphoid tissue has any influence on the response of the immature ovaries to chorionic gonadotrophin. However, the atrophy of the ovaries caused by prolonged treatment with testosterone propionate was shown not to interfere with their normal response to chorionic gonadotrophin (Selye, 1941).

The partial or total inhibition of the formation of corpora lutea may throw some light on the mechanism of abortion produced by carcinogens in pregnant animals. Wolfe and Bryan (1939) showed that daily subcutaneous injection of 5·0 mg. of 1:2:5:6-dibenzanthracene or 3:4-benzpyrene into pregnant rats caused death and resorption of the foetuses. Strong and Hollander (1947) observed a high mortality of embryos of C3H and A mice injected intraperitoneally with 1·0 mg. of methylcholanthrene. These authors suggested that a disturbed vitamin A metabolism, as shown by Goerner and Goerner (1943), may be the cause of the mortality of the embryos. As no symptoms of vitamin A deficiency were observed in the treated females or their offspring, Strong and Hollander assumed that some unknown influence on the corpus luteum may have been the cause of the abortions. The present results show that methylcholanthrene inhibits to a greater or less extent the formation of corpora lutea, and this may be helpful in explaining the mechanism of the abortion following the injection of carcinogenic hydrocarbons. It must be emphasized, however, that the inhibitory influence of methylcholanthrene on the formation of corpora lutea was observed only in immature mice with artificially induced corpora lutea. In adult females injected subcutaneously with 1·0 mg. of methylcholanthrene corpora lutea were found, and such mice became pregnant, and gave birth to normal litters. The influence of intraperitoneal injection of larger doses of methylcholanthrene on corpora lutea has not been investigated, but Lees (1937*a*, 1937*b*, 1937*c*) found no lasting interference with normal oestrous cycles in rats injected with 20·0 mg. of 1:2:5:6-dibenzanthracene.

A growth-inhibitory influence of methylcholanthrene on the comb in chicks was recently described by Hertz and Tullner (1947), who employed testosterone propionate as the growth-promoting agent. A marked inhibition of growth and certain abnormalities of the comb in the carcinogen-treated birds were observed.

The influence of other carcinogenic and non-carcinogenic hydrocarbons on the gonadotrophic reaction of the immature ovaries has not yet been investigated. By this means a relation between the inhibitory influence on the ovary and the carcinogenic properties of the hydrocarbons might be brought to light.

SUMMARY.

(1) 20-Methylcholanthrene was found to exert an inhibitory influence on the action of chorionic gonadotrophin on the ovaries of immature CBA and Strong A mice.

(2) Immature CBA mice injected subcutaneously with 2·0–6·0 mg. or intraperitoneally with 1·0–1·5 mg. of 20-methylcholanthrene, and given chorionic gonadotrophin four to eight days later, showed no blood points and various degrees of inhibition of luteinization. A similar inhibition of luteinization was observed in Strong A mice, but the absence of blood points in these mice could not be considered significant.

(3) The inhibitory influence of 20-methylcholanthrene on follicle haemorrhage and luteinization became apparent in a number of mice before the carcinogen showed its influence on the weight of the body as a whole.

(4) There is no evidence as to whether this inhibition is caused by the direct action of the carcinogen on the ovaries or indirectly through the pituitary gland.

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ERRATUM.

Dr. KHANOLKAR reports that the figure 12·7 given for carcinoma of the cervix as percentage of all carcinomas in Indian Christian women in Tables XXIII (p. 192) and XXXIV (p. 208) in the paper "The Racial and Social Incidence of Cancer of the Uterus" (this Journal, Vol. II, pp. 177–212) was an error in his typescript. The correct figure is 30·8. The allusions to the erroneous figure on pp. 193, 210 require deletion.—E. L. K.

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SURVEY OF PAPERS.

BADGER reviews the literature on the relationship between carcinogenic activity and chemical constitution in the carcinogenic hydrocarbons. He discusses the effects on activity of the introduction of methyl, alkyl and other substituents in hydrocarbons, and finds that carcinogenic activity is not related to chemical reactivity, and is only partially related to the shape and size of the molecule and to the electronic charge on the phenanthrene-type double bond present in most carcinogens (p. 309).

MITCHELL finds that tetra-sodium 2-methyl-1:4-naphthohydroquinone diphosphate, a synthetic vitamin K substitute, produces a small but useful improvement in the palliative results of X-ray therapy in some cases of advanced cancer and an increase in survival time in cases of inoperable carcinoma of the bronchus (p. 351).

ROBINSON has used a polarographic method for the examination of a number of sera from patients with malignant and non-malignant conditions. Repeated examination of sera from patients with carcinoma of the prostate gives a good indication of the effectiveness of therapy with synthetic oestrogens (p. 360).

LASNITZKI AND WILKINSON have studied the effect of certain acridine compounds on growth and mitosis in cultures of chick fibroblasts. The effect on mitosis resembles that of small doses of radiation (p. 369).

SELBIE, ROBINSON AND SHOPE find that a strain of Shope papilloma which has been adapted to serial passage in the domestic rabbit has lost its adaptation after a single passage in the cottontail rabbit. Their findings are consistent with the view that infection is achieved in the domestic rabbit with much less multiplication of the virus than in the cottontail rabbit (p. 375).

SELBIE AND THACKRAY have examined lung adenomas produced in CBA mice by the administration of urethane. They find that the tumours arise from alveolar epithelium and are not necessarily preceded by pneumonic changes (p. 380).

FLAKS finds that large doses of testosterone propionate delays the appearance of tumours induced in mice by the subcutaneous injection of methylcholanthrene. It is suggested that testosterone may interfere with the malignant transformation of the normal cell under the influence of methylcholanthrene (p. 386).

FLAKS finds that methylcholanthrene has an inhibitory influence on the action of chorionic gonadotrophin on the ovaries of immature mice (p. 395).